# (*R*)-Indolelactyl-CoA dehydratase, the key enzyme of tryptophan reduction to indolepropionate in *Clostridium sporogenes*

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*Für den gläubigen Menschen steht Gott am Anfang, für den Wissenschaftler am Ende aller seiner Überlegungen.*

*Max Planck*

献给最亲爱的爸爸妈妈

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*ABBREVIATIONS*

| | |
|---|---|
| DMSO | Dimethylsulfoxide |
| DNP | 2,4-Dinitrophenol |
| DTT | Dithiothreitol |
| ESI-MS | Electrospray-Ionization-Mass Spectrometry |
| FPLC | Fast Protein Liquid Chromatography |
| LC-MS | Liquid Chromatography Mass Spectrometry |
| Mops | 3-(N-morpholino) propanesulfonic acid |
| MALDI-TOF-MS | Matrix Assisted Laser Desorption Ionization-Time Of Flight-Mass Spectrometry |
| NMR | Nuclear Magnetic Resonance |
| OD | Optical Density |
| Tris | 2-Amino-2-hydroxymethylpropane-1,3-diol |
| UV-vis | Ultraviolet visible |

# *ZUSAMMENFASSUNG*

In dieser Arbeit wurde der Weg der Fermentation von Tryptophan zu Indolpropionat in *Clostridium sporogenes* sowie das Schlüsselenzym Indollactat-Dehydratase untersucht. Die Zwischenprodukte und Endprodukte der Fermentation wurden mittels Massenspektrometrie nachgewiesen. Tryptophan disproportioniert oxidativ über 3-Indolpyruvat zu 3-Indolacetat und reduktiv über (*R*)-3-Indollactat und (*E*)-3-Indolacrylat zu 3-Indolpropionat (IPA). IPA, das im menschlichen Darm gebildet wird, gelangt über das Blut ins Gehirn, wo es Sauerstoffradikale (ROS) abfängt und dadurch vor der Alzheimer Krankheit schützen kann. Die Aktivitäten der Enzyme der Tryptophan-Reduktion (Tryptophan Transaminase, Indollactat Dehydrogenase, Indollactat Dehydratase und Indolacrylat Reduktase) wurden in im Rohextrakt von Tryptophan-Kulturen nachgewiesen. In ganz analoger Weise wird Phenylalanin von *C. sporogenes* über (*R*)-Phenyllactat und (*E*)-Zimtat zu Phenylpropionat reduziert. Die Arbeit hat sich besonders auf das Schlüsselenzym Indollactyl-CoA Dehyratase mit einem ungewöhnlichen radikalischen Umpolungs-Mechanismus fokussiert. Die Dehydratase katalysiert die radikalische *syn*-Eliminierung eines nicht aktivierten Protons in der β-Position und einer OH-Gruppe in der α-Position des Thioestercarbonyls. Erstaunlicherweise zeigt die Dehydratase aus der Tryptophan Kultur eine höhere spezifische Aktivität mit Indollactyl-CoA als mit Phenyllactyl-CoA, während mit der Dehydratase aus der Phenylalanin Kultur das umgekehrte der Fall ist. Beide Enzyme bestehen aus einem Komplex mit je drei Untereinheiten. Mittels Peptide MALDI-TOF Fingerprinting und Nano LC-MS wurden die Untereinheiten als eine CoA-Transferase (FldA) und eine heterodimere Dehydratase (FldBC) identifiziert. Die Peptid Sequenzen der drei Untereinheiten aus Tryptophan sowie Phenylalanin Kulturen sind identisch und werden von denselben Genen *fldABC* kodiert. Die rekombinante FldBC aus *E. coli* zeigt sogar eine noch höhere Aktivität als die native Dehydratase FldABC. Die Untereinheit FldB der nativen Dehydratasen hat eine um 2 kDa kleinere molekulare Masse als vom Gen *fldB* vorausgesagt, wobei die N- und C-Termini erhalten sind, während ein internes 4,3 kDa Peptid beim Peptide-Fingerprinting nicht nachgewiesen werden konnte. Modellierungsversuche von FldBC mit der bekannten Struktur der 2-Hydroxyisocaproyl-CoA-Dehydratase zeigen, dass die 2. Hälfte des 4,3 kDa Peptids eine Schleife bildet, die durch posttranslationales Spleißen herausgeschnitten werden könnte. Zudem induzieren Phenylalanin und Tryptophan möglicherweise unterschiedliche Schnittstellen, mit denen man die ober erwähnten verschiedenen spezifischen Aktivitäten erklären könnte.

# *SUMMARY*

In this thesis the pathway of the fermentation from tryptophan to indolepropionate in *Clostridium sporogenes* and the key enzyme indolelactyl-CoA dehydratase were investigated. The intermediates and the end product were detected via mass spectrometry. Tryptophan disproportionates oxidatively via 3-indolepyruvate to 3-indoleacetate and reductively via (*R*)-3-indolelactate and (*E*)-3-indoleacrylate to 3-indolepropionate (IPA). IPA, which is formed in the human intestine, is transferred via the blood to the brain. In the human brain IPA scavenges reactive oxygen species (ROS) and thus protects from Alzheimer's disease. The activities of the enzymes from the reductive branch of tryptophan fermentation (tryptophan transaminase, indolelactate dehydrogenase, indolelactate dehydratase and indoleacrylate reductase) were determined in the cell-free extract. Similarly, phenylalaine is reduced by *C. sprogenes* via (*R*)-phenyllactate and (*E*)-cinnamate to 3-phenylpropionate. Further this thesis focused on the key enzyme indolelactyl-CoA dehydratase, which catalyzes a very unusual *syn*-elimination of a non-activated proton at the β-position and an OH-group at the α-position of the thioester carbonyl by employing radical intermediates. Surprisingly the dehydratase purified from cultures growing on tryptophan shows a higher specific activity with indolelactyl-CoA than with phenyllactyl-CoA. The reverse was the case with the dehydratase purified from cultures growing on phenylalanine. Both native dehydratases consist of three subunits, which were identified via peptide MALDI-TOF fingerprinting and Nano LC-MS as the CoA-transferase (FldA) and the heterodimeric dehdratase (FldBC). The peptide sequences of the three subunits from the culture growing on tryptophan as well as that on phenylalanine are identical and derived from the same genes *fldABC.* The recombinant FldBC produced in *E. coli* shows even a higher specific activity than the native dehydratase FldABC. It has been observed that the molecular mass of the subunit FldB of the native dehydratase is around 2 kDa smaller than that calculated from the gene. Peptide fingerprinting showed that the N- and C-termini remained, but one internal 4.3 kDa peptide could not be detected. The predicted model of FldBC based on the structure of 2-hydroxyisocaproyl-CoA dehydratase shows that the C-terminal half of this peptide could have been cut off by posttranslational splicing. Probably tryptophan and phenylalanine induce slightly different splicing positions, which result in different specific activities of the dehydratase.

# *INTRODUCTION*

## *1. The role of gastrointestinal microbiota metabolites*

The human intestine is colonized by trillions of widely diverse microbes, which perform different functions to interfere the host. For a long time clinicians and researchers have recognized that the metabolites of gastrointestinal microbiota influence the central nervous system. This interaction is called 'gut-brain axis' that physiologically connects the gut, liver, muscle and brain. The metabolites from aromatic amino acids, which are contributed by the gut bacterium *Clostridium sporogenes*, have potential biological functions. Phenyl derivatives (phenylacetate, phenylpropionate, 3-hydroxyphenylpropionate and 4-hydroxyphenylpropionate) are elevated in colorectal cancer. Indole derivatives (indoleacetate, indole, and 3-indolepropionate) protect against stress-induced lesions in the GI (human gastrointestinal) tract, increase expression of anti-inflammatory genes and they are implicated in GI pathologies, brain-gut axis and a few neurological conditions [1-3].

The energy requirement of all organisms depends on chemical work mediated in Eukarya by oxidative metabolism located mainly in the mitochondria, which also produce oxygen radicals. Due their high chemical reactivity oxygen radicals can cause unspecific but severe damages leading to diseases like Alzheimer's disease. Alzheimer's disease is diagnosed very often in people over 65 years of age. The symptoms include confusion, irritability and aggression, mood swings, language breakdown, long-term memory loss. Radical oxygen species can induce the proteolytic digestion of a nerve cell trans-membrane protein, amyloid precursor protein (APP) into smaller fragments called as beta-amyloid protein. The accumulation of those beta-amyloid proteins is the major pathogenic event of Alzheimer's disease, because they deposit outside neurons and build up between nerve cells leading to brain degeneration. The quenching of these oxygen radicals is supposed to slow brain degeneration. In view of therapeutic strategies, indole-type substances are preferred. 3-Indolepropionate (IPA) as a unique potent antioxidant shows excellent oxygen radical scavenger effect for counteracting the toxic effect of amyloid β-protein and in contrast with other antioxidants. IPA is not converted to reactive intermediates with pro-oxidant activity. IPA is an endogenous substance found in the plasma and due to its amphiphilic side chain IPA can pass the blood-brain barrier of humans. The hydroxyl radical, the most reactive oxygen species, oxidizes IPA to the indolyl cation radical, which in turn is oxidized by the superoxide anion, another oxygen-derived radical, to kynuric acid. The exact physiological role (and possible therapeutic use) of IPA awaits further investigation. [4-6]

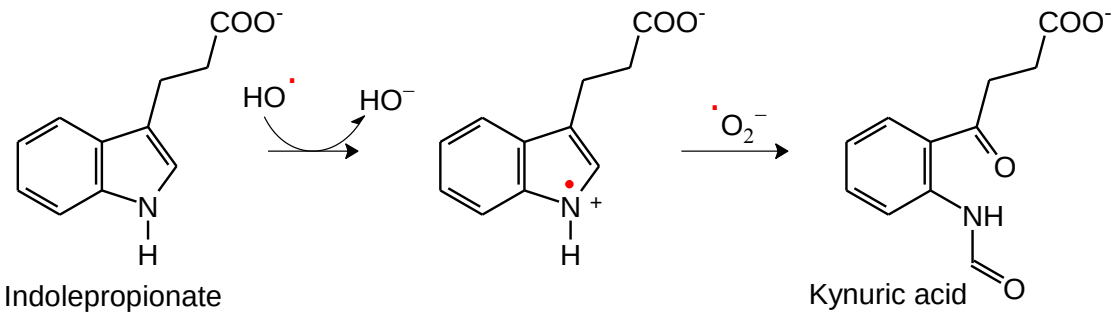



**Fig. 2** The mechanism of reduction of oxygen radicals by IPA

A deeper understanding of the metabolic degradation of tryptophan could manipulate the metabolite production in gut to improve the therapeutic strategies for neuron disease. It has been shown by Elsden that all three aromatic amino acids could be reduced by *Clostridium sporogenes* to phenylpropionate, indolepropionate and hydroxyphenylpropionate, but the oxidation products phenylacetic acid and indoleacetic acid were not found (Table 1) [7]. In the publication of Wikoff et al. [6] it has been also shown that the production of IPA was completely dependent on the presence of *C. sporogenes* and could be established by colonization with this bacterium. They proposed that similar to the degradation of tryptophan in *E. coli*, tryptophan from the food source was first degraded to single inole by tryptophanase. The product indolepropionate could be synthesized from indole in *C. sporogenes* and further transferred into the blood. But the study on the degradation pathway of phenylalanine by *C. sporogenes* by S. Dickert & W. Buckel showed that the fermentation started with a transamination to phenylpyruvate, which further was reduced via phenyllactate and phenylacrylate to phenylpropionate. In this fermentation phenylalanine also acted as electron doner and was oxidized to phenylacetate [8]. Based on the structural similarity between tryptophan and phenylalanine, I suppose that the reduction of tryptophan to indolepropinate by *C. sporogenes* is similar to that of phenylalanine to phenylpropionate.

**Table 1** The end products of the metabolism of aromatic amino acids by clostridia. [7]

| Species | Strain | Products formed µmoles/ml | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Phenyl acetic | Phenyl propi-onic | Phenyl lactic | Phenol | HO-phenyl acetic | HO-phenyl propi-onic | *p*-Cresol | Indole | Indole acetic | Indole propi-onic |
| *Clostridium sporogenes* | NCIB 10696 | 0 | 13.1 | 0 | 0 | 0 | 7.4 | 0 | 0 | 0 | 2.3 |
| *Clostridium botulinum A* | NCIB 10640 | 0 | 13.0 | 0 | 0 | 0 | 8.0 | 0 | 0 | 0 | 1.6 |
| *Clostridium botulinum B* | NCIB 10657 | 0 | 11.8 | 0 | 0 | 0 | 8.2 | 0 | 0 | 0 | 2.4 |
| *Clostridium caloritolerans*[a] | NCIB 9360 | 0 | 11.2 | 0 | 0 | 0 | 3.8 | 0 | 0 | 0 | 1.6 |
| *Clostridium mangenoti* | NCIB 10639 | 0 | 2.3 | 2.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clostridium ghoni* | NCIB 10636 | 0 | 6.4 | 4.9 | 0 | 0 | 0 | 0 | 0.4 | 0 | 0 |
| *Clostridium bifermentans* | NCIB 10716 | 0.8 | 9.8 | 2.3 | 0 | 0 | 0 | 0 | 0.6 | 0 | 0 |
| *Clostridium sordellii* | NCIB 10717 | 0.8 | 5.9 | 0.5 | 0 | 0 | 0 | 0 | 1.2 | 0 | 0 |
| *Clostridium difficile* | NCIB 10666 | 4.8 | 0.8 | 0 | 0 | 0.5 | 0 | 2.3 | 0 | 0.6 | 0 |
| *Clostridium sticklandii* | NCIB 10654 | 2.6 | 0 | 0 | 0 | 0.8 | 0 | 0 | 0 | 0.5 | 0 |
| *Clostridium lituseburense* | NCIB 10637 | 0.6 | 0 | 0 | 0 | 0.6 | 0 | 0 | 0 | 0.2 | 0 |
| *Clostridium subterminale* | NCIB 9384 | 0.5 | 0 | 0 | 0 | 0.7 | 0 | 0 | 0 | 0.2 | 0 |
| *Clostridium putrefaciens* | NCIB 9836 | 0.5 | 0 | 0 | 0 | 0.4 | 0 | 0 | 0 | 0.2 | 0 |
| *Clostridium propionicum* | NCIB 10656 | 0.1 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 |
| *Clostridium malenomenatum* | ATCC 25776 | 0 | 0 | 0 | 0.3 | 0 | 0 | 0 | 0.4 | 0 | 0 |
| *Clostridium limosum* | NCIB 10638 | 0 | 0 | 0 | 2.6 | 0 | 0 | 0 | 9.8 | 0 | 0 |
| *Clostridium lentoputrescens* | NCIB 10629 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 3.3 | 0 | 0 |
| *Clostridium tetani* | NCIB 10628 | 0 | 0 | 0 | 1.9 | 0 | 0 | 0 | 3.9 | 0 | 0 |
| *Clostridium tetanomorphum*[a] | NCTC 500 | 0 | 0 | 0 | 5.0 | 0 | 0 | 0 | 0.6 | 0 | 0 |
| *Clostridium cochlearium* | ATCC 17787 | 0 | 0 | 0 | 3.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clostridium histolyticum* | NCIB 503 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clostridium aminovalericum* | NCIB 10631 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clostridium sporospheroides* | NCIB 10672 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## 2. *Fermentation of amino acids and Stickland-reaction*

Aerobes and anaerobes usually convert valuable nutrients to the corresponding α-oxo acids and oxidize them further via the citric acid cycle to $CO_2$. In the absence of electron acceptors like oxygen, nitrate and sulfate only clostridia, fusobacteria and few of other anaerobes can use amino acids as energy sources. The substrate spectrum for clostridia is very broad and includes a wide range of naturally occurring compounds; they play a major role in the degradation of organic material in the soil, intestines of human and animals and other environments. The anaerobic food chain, in which clostridia participate, starts with hydrolysis of polymers from food such as proteins and carbohydrates to sugars, small peptides and single amino acids. Amino acids are further fermented to ammonia, $CO_2$, short chain fatty acids and molecular hydrogen and finally to methane and $CO_2$. (Fig. 3) *Clostridium sporogenes* can ferment glucose to a variety of products but its preferred substrates are amino acids, which are fermented by Stickland-reaction [9, 10].

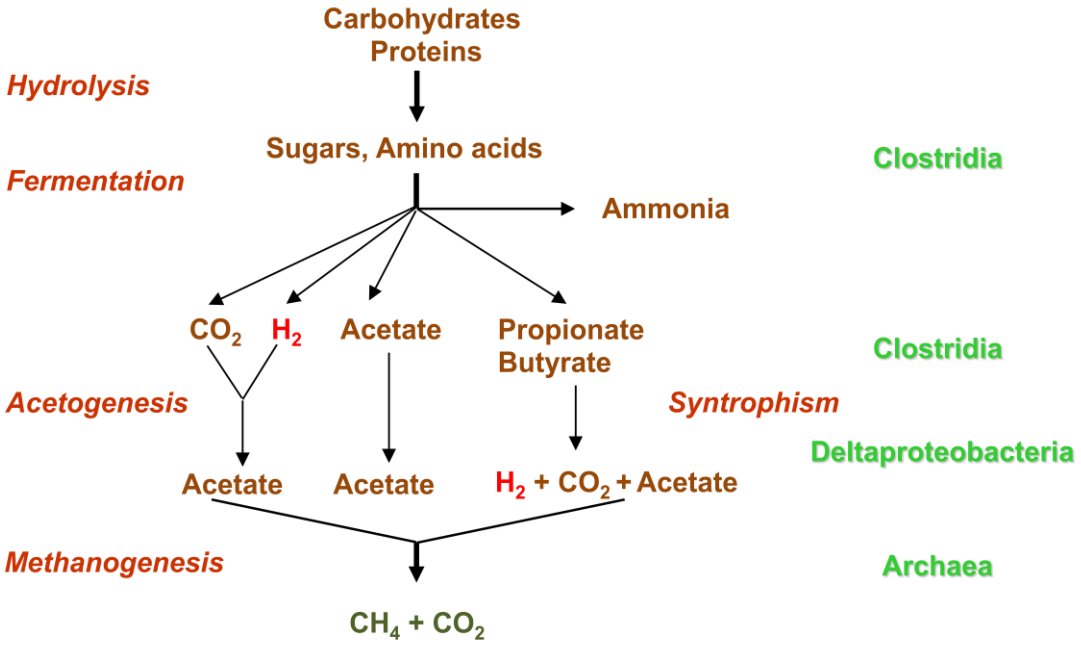



**Fig. 3** Anaerobic food chain fermentation of carbohydrates and proteins

Most fermentations of amino acids by different *Clostridium* stains follow the Stickland-reaction. These obligate anaerobe bacteria do not use molecular oxygen as a final electron acceptor; therefore the oxido-reduction becomes a question in the metabolism. Stickland and Woods discovered the coupled deamination between two amino acids acting as electron donor and acceptor respectively. This chemical reaction was first determined in *C. sporogenes*, which obtained energy and carbon from amino acids as sole sources for growth. Stickland observed that in a suspension of *C. sporogenes* with amino acids (like alanine, valine, leucine and isoleucine) they could provide reducing equivalents to reduce methylene blue and benzylviologen. Later Woods could also show that proline acted as electron acceptor in presence of molecular hydrogen in a suspension of *C. sporogenes*[11]. The theory of Stickland-reaction explains the oxido-reductive reaction of the fermentation of amino acids by anaerobic bacteria. One of the coupled amino acids as electron donor is oxidized to $CO_2$, ammonium and a carboxylic acid, which is one carbon atom shorter than the original. In the same time the other one of the coupled amino acids is reduced as electron acceptor of the partner to carboxylic acid with same length as the original amino acid (Fig. 4). Most of the proteinogenic amino acids could act electron donor as well as electron acceptor in Stickland-reactions; exceptions are isoleucine and valine, which are only oxidized.

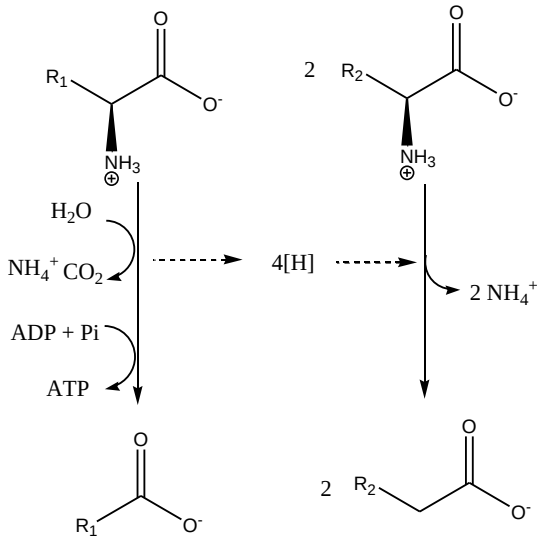



**Fig. 4** Stickland reaction of two oxido-reductive coupled amino acids

## *3. Clostridium sporogenes*

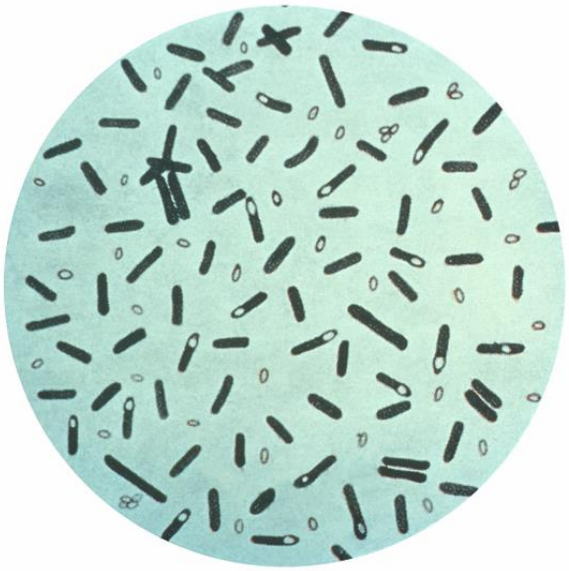


**Fig. 1** *Clostridium sporogenes* (Wikipedia, US Department of Energy. Retrieved 5 September 2011)

*Clostridium sporogenes* is classified on the basis of its ability to ferment amino acids as a proteolytic member of the genus *Clostridium*, order *Clostridales*, family *Clostridiaceae*. Under adverse conditions *C. sporogenes* produces oval, subterminal endospores. A number of clostridia produce exotoxins and are pathogenic for humans and animals, like tetanus from *Clostridium*

*tetani*, gas gangrene from *Clostridium perfingers*, an antibiotic-associated enterocolitis from *Clostridium difficile* and botulism from *Clostridium botulinum*. *C. sporogenes* is very close related to *C. botulinum* Hall strain A, actually *sporogenes* is the name given to the strain of *C. botulinum* that does not produce botulinum neurotoxins. Based on the types of the botulinum neurotoxins (types A to G) *C. botulinum* contains four distinct genetic and physiological groupings. Each of the four groupings has a nonneurotoxinogenic counterpart, e.g. *Clostridium sporogenes* for Group I (proteolytic strain produces the toxins of type A, B or F) and *Clostridium novyi* for Group III (produces toxins of typ C or D). Despite different toxin types Group I strains are highly related to each other and form together with *C sporogenes* a single phylogenetic unit [12] [13]. The nontoxigenic *C. sporogenes* strain ATCC 3857 shares 84-87% of the CDS (coding DNA sequence) with the type strain Hall A of *C. botulinum*. Two gene clusters that encode key enzymes (phenyllactate dehydratase FldAIBC and 2-hydroxyisocaproyl-CoA dehydratase HadAIBC) in the fermentation pathways of phenylalanine and leucine were also identified in the *C. botulinum* genome. The phenyllactate dehydratase gene cluster *fldAIBC*, which is missing in the other sequenced clostrdial genomes, is highly similar (over 95 %) to that of *C. sporogenes* [12].

The bacteria of genus *Clostridium* are usually rod-shaped and Gram-positive. They are found widely in soil, water and the intestinal tracts of humans and other animals. Clostridia are obligate anaerobes, because they are unable to use molecular oxygen as a final electron acceptor and generate their energy solely by fermentation. Some *Clostridium* species are sensitive to oxygen concentrations as low as 0.5%, but most species can tolerant concentrations of 3-5%. *C. sporogenes* is ubiquitous in many natural environments and is of some economic and medical importance.

*C. sporogenes* could be also applied as cancer gene therapy vectors. The hypoxic and necrotic regions are very common in human solid tumors. The anaerobic non- pathogenic strain of genus *Clostridium* could be used a genetically engineered strain to target cancer therapy to tumors. The strategy of the bacterially directed enzyme prodrug therapy uses anaerobic bacteria that have been transformed with an enzyme which can convert a non-toxic prodrug into a toxic drug to kill tumor, like cytosine deaminase (CD) can convert 5-fluorocytosine (5FC) to 5-fluorouracil (5FU) and nitroreductase (NR) can convert the prodrug CB1954 to a DNA cross-linking agent. With the proliferation of the bacteria in the necrotic and hypoxic areas of the tumor the enzyme is produced solely in the tumor and this systemically applied prodrug is metabolized to a toxic drug only in the tumor. The early study shows that the successful transformation of *C. sporogenes*

with the highest reported tumor colonization efficiency with the CD gene and the systemically injected spores of these bacteria express CD only in the tumor. [14]

## 4. *2-Hydroxyacyl-CoA dehydratases and the unusual radical $H_2O$-elimination*

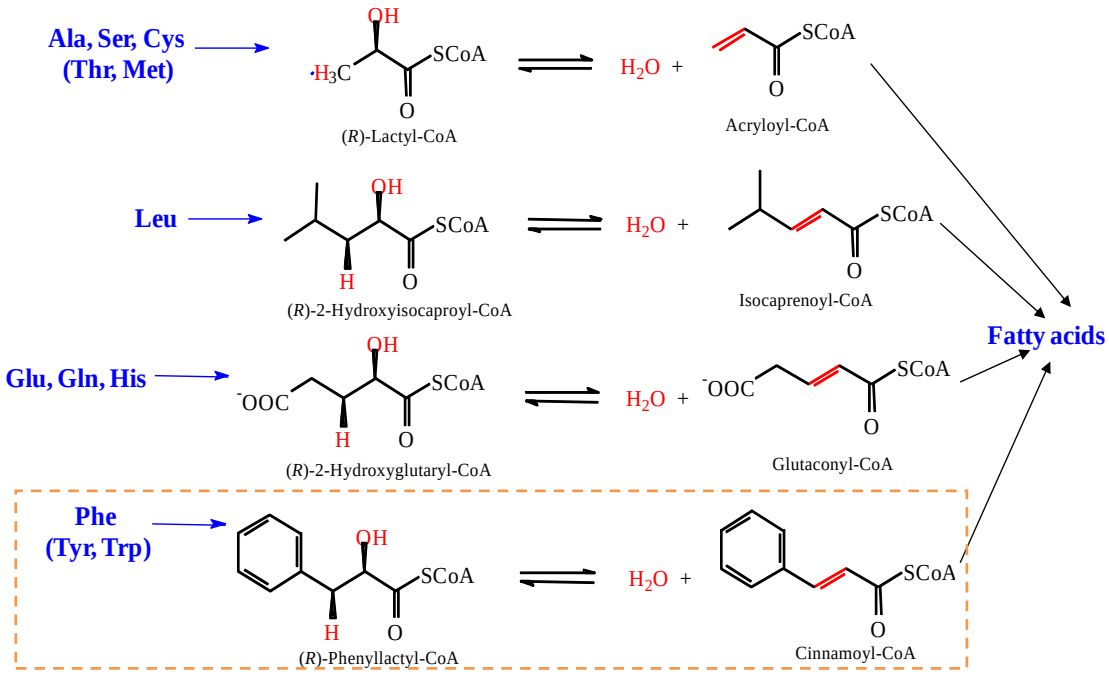



**Fig.5** Dehydration, the key step in the fermentation of amino acids via 2-hydroxyacids by certain clostridia

Twelve of the twenty proteinogenic amino acids can be fermented by different clostridia stains and related organisms via Stickland-reaction to various products like acetate, propionate, butyrate, arylacetates, arylpropionate, ammonia, carbon dioxide and molecular hydrogen (Fig. 5). One amino acid or part of it is oxidized and decarboxylated via the corresponding 2-oxoacid to an "energy-rich" thioester, which leads to energy conservation from the formation of ATP on the substrate phosphorylation level, also to electron conservation to ferredoxin. Another or the same amino acid acts as electron acceptor to be reduced to short chain fatty acid. In this reductive branch of Stickland-reaction all amino acids are reduced via their 2-hydroxyacids as unalterable intermediate. But in contrast to the E2 dehydration of 3-hydroxybutyryl-CoA with *syn*-geometry, the dehydration of 2-hydroxyacyl-CoA like 2-hydroxybutyryl-CoA, actually the $H_2O$-elimation of α-hydroxyl group and β-hydrogen seems not possible. Because the hydrogen at the β-position with p*K* 40 is very challenging to be deprotonated in the biologic environment and the hydroxyl at the α-position adjacent to the carboxyl group is also not able to be expelled (Fig. 6). Therefore

the dehydration of 2-hydroxyacyl-CoA to 2-enoyl-CoA is the mechanistically most difficult and demanding step of the fermentations. Early studies in Wolfgang Buckel´s group showed that the 2-hydroxyacyl-CoA dehydratases are extremely sensitive to oxygen and catalyze a reversible *syn*-elimination of $H_2O$. To carry out the dehydration, the 2-hydroxyacid needs to be first activated to CoA-thioester (Fig. 5).

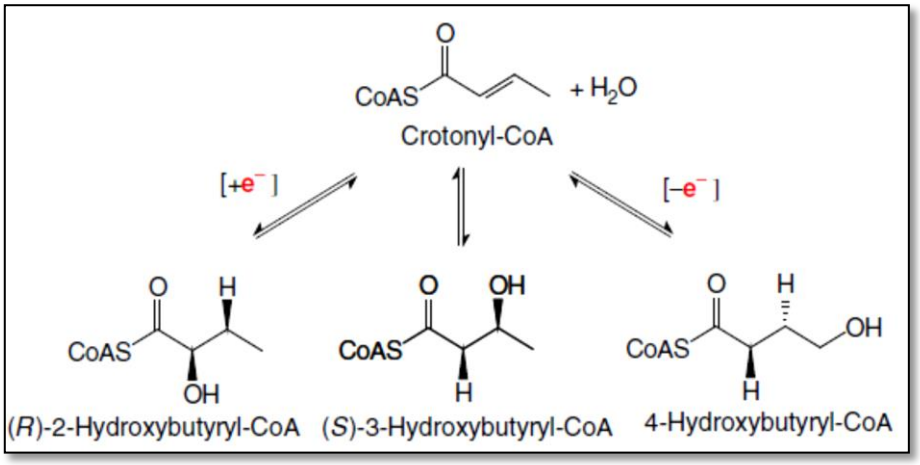



**Fig.6** Three modes of the reversible hydration of crotonyl-CoA, 2-hydroxybutyryl-CoA and 4-hydroxybutyryl-CoA

In nature, radical anions are always used as versatile intermediates to realize those challenging enzymatic reactions. In 1951 Feodor Lynen has already recognized that the properties of CoA-thioesters are similar to ketones. In chemistry α-hydroxyketones can be reduced to unsubstituted ketones by an inorganic one-electron donors like $Zn^0$, $Cr^{2+}$ or $Sm^{2+}$. One electron is transferred to the ketone and causes a polarity inversion of the carbon with the reaction name “Umpolung”, and then the original electrophilic carbonyl group converts to a nucleophilic ketyl, which is stabilized by the interacting between the $\pi^*$-$2p_z$ (C,O) antibonding orbital of carbon and oxygen. A ketyl eliminates the adjacent leaving group to yield a neutral radical (Fig.7). The discovery of five different anaerobic reactions: 1) the reversible *syn*-dehydration of (*R*)-2-hydroxyacyl-CoA to (*E*)-enoyl-CoA; 2) the reversible dehydration of 4-hydroxybutyryl-CoA to crotonyl-CoA; 3) the oxidation of phenylacetyl-CoA to mandeyl-CoA; 4) the reduction of benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA; 5) the reduction of 4-hydroxybenzoyl-CoA to benzoyl-CoA, promted W. Buckel and R. Keese to use the ketyl as a guide to propose a new radical mechanism. This proposal explains very well the challenging dehydration from 2-hydroxyacyl-CoA to 2-enoyl-CoA based on the model radical reduction of an α-hydroxyketone to an unsubstituted ketone (Fig. 7).

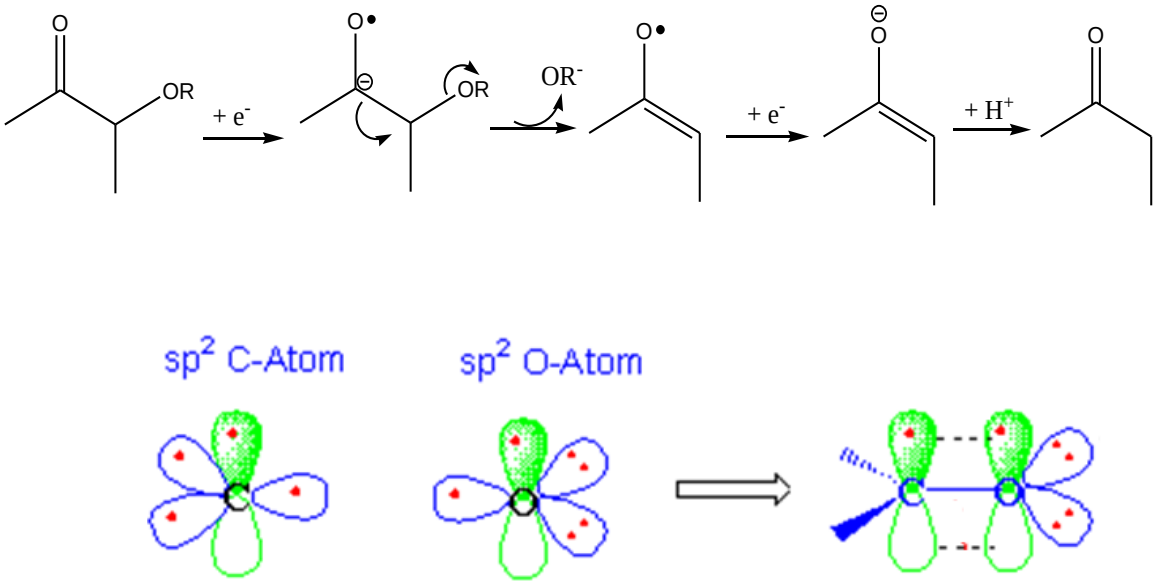



**Fig.7** The radical reduction of α-hydroxylketone to unsubstituted ketone by one electron

The purification and characterization of 2-hydroxyacyl-CoA dehydratase from the early study also showed that all dehydratases were composed of two iron-sulfur-cluster containing subunits (α, β). To start the reaction they require activators with an iron-sulfur-cluster, ATP, $Mg^{2+}$ and a one-electron reducing agent like dithinoite or Ti(ш) citrate in vitro or reduced ferredoxin in vivo (Table 2).

**Table 2** Purified dehydratases and their cofactors and activators [15]

| Organism | Substrate | Dehydratase and cofactors | Activator |
|---|---|---|---|
| *Acidaminococcus fermentans* | (*R*)-2-hydroxylglutaryl-CoA | HgdAB, FMN, riboflavin, [4Fe-4S] | HgdC |
| *Clostridium symbiosum* | (*R*)-2-hydroxylglutaryl-CoA | HgdAB, FMN, riboflavin, [4Fe-4S] | HgdC |
| *Fusobacterium nucleatum* | (*R*)-2-hydroxylglutaryl-CoA | HgdABC, riboflavin, [4Fe-4S] | HgdD |
| *Clostridium propionicum* | (*R*)-lactyl-CoA | LcdAB, FMN, riboflavin, [4Fe-4S] | LcdC |
| *Clostridium difficile* | (*R*)-2-hydroxyisocaproyl-CoA | HadBC 2[4Fe-4S] | HadI |
| *Clostridium sporogenes* | (*R*)-phenyllactyl-CoA | FldBC [4Fe-4S] | FldI |

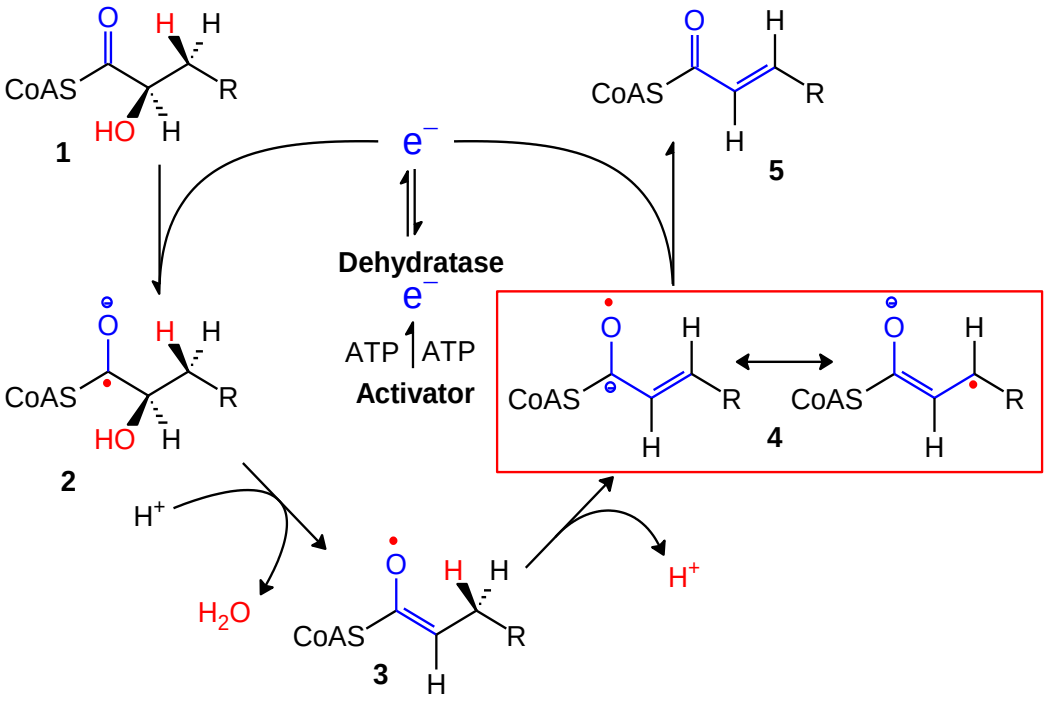



**Fig.8** The unusual radical dehydration of 2-hydroxyacyl-CoA to enoyl-CoA (1: (*R*)-2-hydroxyacyl-CoA; 2: ketyl; 3: enoxy radical; 4: allylic ketyl; 5: enoyl-CoA)

Fig.8 shows the detailed mechanism of the dehydration of 2-hydroxycayl-CoA to enoyl-CoA. The two subunits, actual the two contained [4Fe-4S] clusters resist the reduction direct by conventional reducing agents in vitro with Ti(ш) citrate (-700 mV) or in vivo ferredoxin (-420 mV), that indicates a low redox-potential (< -700 mV) of the dehydratase. The activator should amplify the reductive power of one electron from ca. -300 mV to about -900 mV. This process is speculated that in the presence of ATP and $Mg^{2+}$ the helix-cluster-helix-angle of the activator opens from 150° to 180° and shoots one electron into the dehydratase, similar to shooting an arrow by an archer´s bow (Fig. 9). J. Kim could already show that aluminum tetrafluoride together with ADP ($AlF_4^-$ * ADP) inhibited the activation by forming an isolable complex between dehydratase and activator [16-19].

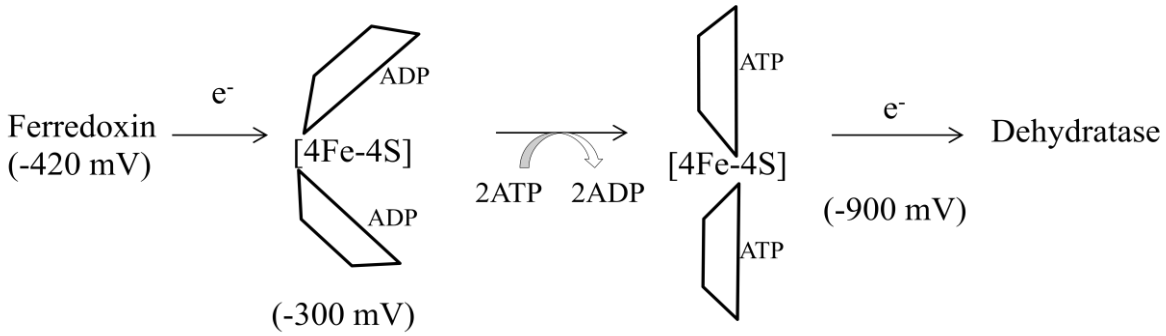



**Fig.9** Activation of the 2-hydroxyacyl-CoA dehydratase by electron transfer.

One single electron is transferred from a reducing agent to the activator. After a conformational change the activator shoots the electron into the dehydratase and further to the thioester of the 2-hydroxyacyl-CoA. The partial positive charge of the carbonyl is reversed by `Umpolung` to a substrate-derived ketyl, which is able to expel the α-hydroxyl. The now much more acidic hydrogen at the β-position (p*K* = 14) of the resulting enoxy radical [20] is deprotonated by a base and yields a product-related allylic ketyl, which is resonance stabilized. The final product 2-enoyl-CoA is formed by returning the electron from the ketyl to the dehydratase for the next turnover (Fig. 8). In 2008 this proposed mechanism has been supported by the identification of the 2-isocaprenoyl-CoA-related allylic ketyl via electron paramagnetic resonace spectroscopy (EPR) [16].

Later the 2-hydroxyisocaproyl-CoA dehydratase together with its substrate was co-crystallized and the structure was solved. The structure helped to get more detail and understanding for this unprecedented mechanism. Each subunit (α and β) contains one [4Fe-4S] cluster. The cluster of the β-subunit acts as electron store and the substrate binding site locates at the cluster of the α-subunit. The carbonyl oxygen of the thioester coordinates the unique iron atom of the [4Fe-4S] cluster replacing the hydroxyl ligand. The carbonyl oxygen also coordinates Glu55, which is highly conserved in 2-hydroxyacyl-CoA dehydratases and the carboxylate group of Glu55 is oriented toward the substrate by Ser37. An inner sphere electron transfer from $Fe_a$ to the carbonyl carbon of the thioester results in the ketyl. The expelled α-hydroxyl acts as base to depronate the β-*Si*-proton to the allylic ketyl, which finally returns the electron to the [4Fe-4S] cluster (Fig. 10a and b) [21].

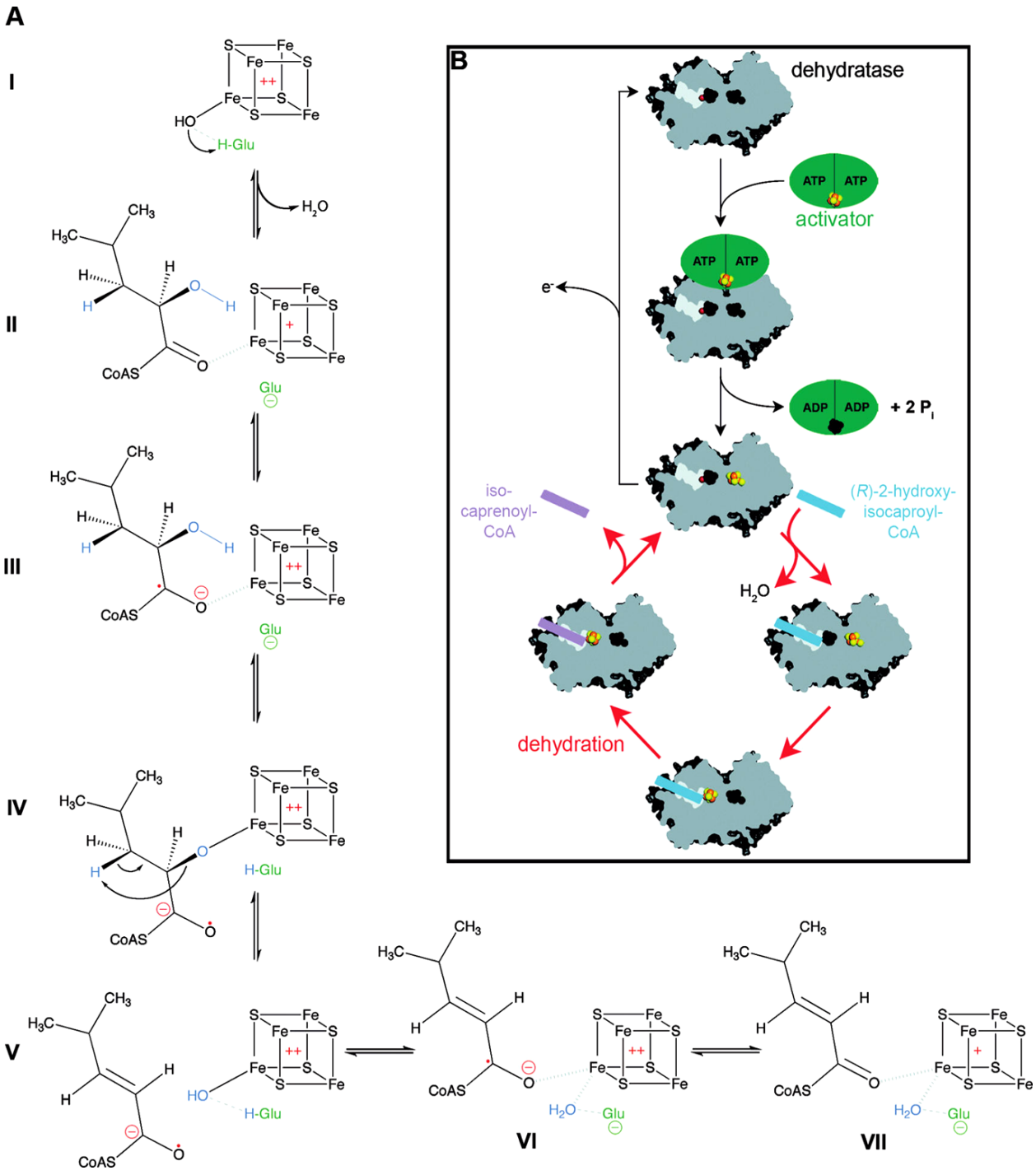



**Fig. 10a** Structure-based reaction mechanism [21].

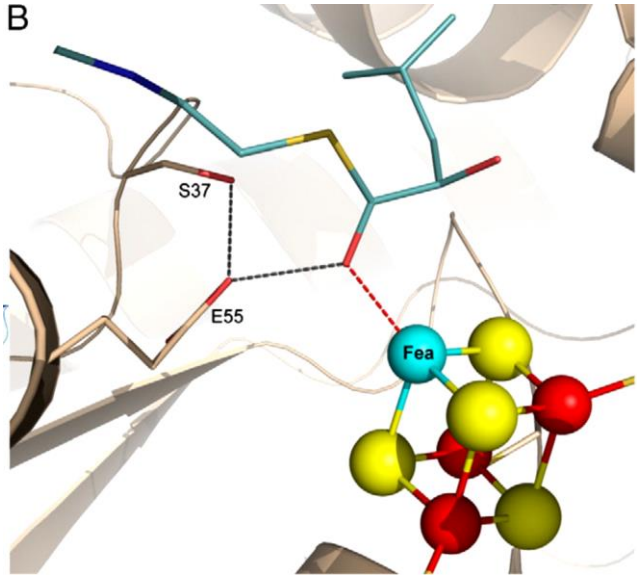



**Fig. 10b** The substrate binding mode in the α-subunit of 2-hydroxyisocaproyl-CoA dehydratase.[15]

## 5. *Family III CoA-transferases*

The conversion of a (*R*)-2-hydroxy acid to an electron withdrawing CoA-thioester is essential for the unusual radial dehydration; therefore CoA transferases are necessarily present in this type of amino acid fermentation pathways. The genes coding for CoA-transferases are located upstream of those coding for the corresponding 2-hydroxycayl-CoA dehydratases. It has been established that propionate CoA-transferase from *C. propionicum* and glutaconate CoA-transferase from *A. fermentans*, *C. symbiosum* and *Fusobacterium nucleatum* are members of family I CoA-transferases. In contrast, phenyllactate CoA-transferase from *C. sporogenes* and *C. botulinum* as well as 2-hydroxyisocaproate CoA-transferase from *C. difficile* belong to family III CoA-transferases. The enzymes of this family use a catalytic mechanism via a ternary complex without intermediates covalently bound to the enzyme (Fig. 11a). Acid-$R_2$ and the CoA-donor-$R_1$ first bind non-covalently to the enzyme to form an anhydride whereby the released $CoAS^-$ stays at the enzyme. A re-attack of the $CoAS^-$ at the other acyl group of Acid-$R_2$ affords acid-$R_1$ and the new CoA-thioester, both of which release the enzyme. The dehydration of carnitine to crotonobetaine occurs also on the CoA-ester level and is most likely identical to the dehydration of 3-hydroxyacyl-CoA to 2-enoyl-CoA. The CoA-transferase family III is also named as 'CaiB/BaiF'. Carnitine CoA-transferase (CaiB) from *E. coli* and *Proteus* sp. catalyses the reversible transfer of CoA between (*R*)-carnitine and butyrobetainyl-CoA and crotonobetainyl-CoA. The presence of a gene coding for a CoA-ligase (CaiC) indicates that catalytic amounts of these CoA-thioesters are required to start the reaction and prevent depletion of the CoA-thioester

pool by unspecific hydrolysis (Fig. 11b) [22]. The phenyllactate CoA-transferase (FldA) from *C. sporogenes* forms with phenyllactyl-CoA dehydratase (FldBC) an enzyme complex, which participates in the fermentation of phenylalanine. FldA shares 24 % with CaiB and 45% sequence identity with 2-hydroxyisocaproate CoA-transferase (HadA) [8, 22].

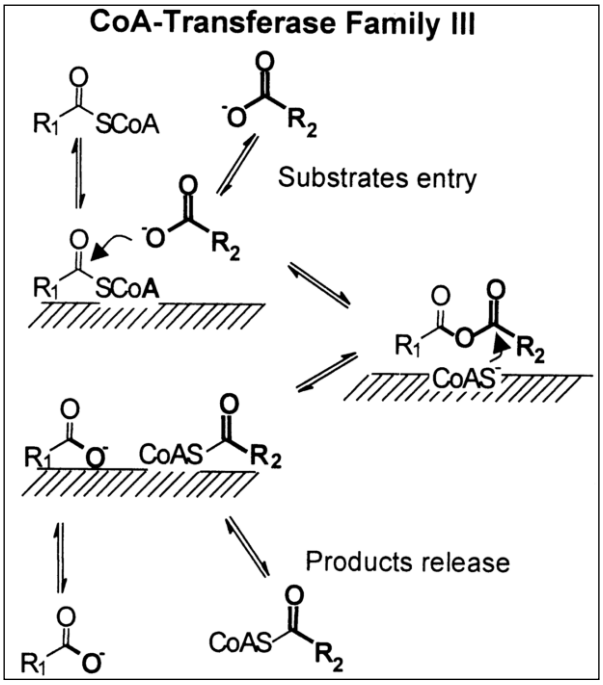



**Fig. 11a** Mechanism of CoA transferase family III [22]

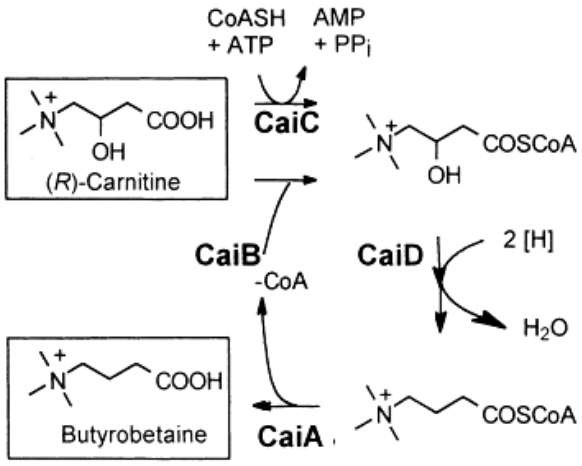



**Fig. 11b** Mechanism of anaerobic carnitine metabolism [22]

## 6. *Aim of thesis*

My work focuses on the fermentations of the aromatic amino acids in *C. sporogenes*, especially on that of tryptophan. In this thesis the pathway of fermentation of tryptophan and the intermediates will be determined. The aim of this research is to get a deeper understanding of the metabolic degradation of tryptophan in the human intestine and to manipulate metabolite production in the gut for improving the therapeutic strategies for neural diseases. The enzymes of the mechanistically most demanding step, the dehydration of aryllactate to arylacrylate, will be purified and characterized. The gene cluster of phenyllactyl-CoA dehydratase and its activator (*fldABCI*) in *C. sporogenes* was already identified. This gene cluster was also found in *C. botulinum* (in contig 174) with ≥ 95% sequence identity. The organism contains two additional open reading frames (ORFs) (in contig 105) that are similar to the gene cluster *fldABCI* and have around 50% amino acid identities to FldABC [23]. Based on the high identity between those two genomes, *C. sporogenes* certainly also contains these two ORFs (Fig. 12). The dehydratases, which participate in the fermentations of tryptophan and tyrosine, could be encoded by these two dehydratase gene clusters found in *C. botulium* or by *fldABCI*, that is to say, all three aryllactyl-CoA dehydratases may be encoded by the same gene cluster. The molecular properties and the substrate specificities of the dehydratses after growth of *C. sporogenes* either on phenylalanine or on tryptophan and will be investigated.

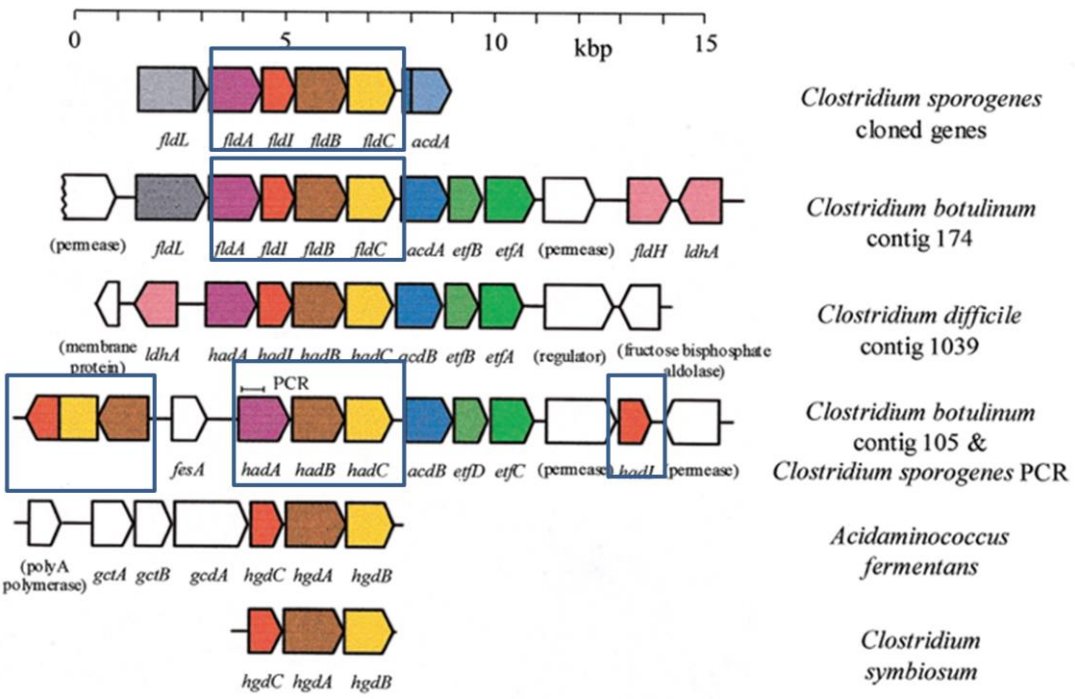



**Fig. 12** The arrangements of genes coding for 2-hydroxy acid dehydratases. [23]

# *MATERIALS AND METHODS*

## *1. Chemicals, biochemichals and reagents*

All chemicals and biochemicals were purchased, if not mentioned separately in the text, from Sigma-Aldrich (Deisenhofen, Germany), Fluka (Neu Ulm), Roth (Karlsruhe, Germany), Merck (Darmstadt, Germany), Roche (Mannheim, Germany), AppliChem, MP biomedicals (USA). The materials for molecular biology were from Fermentas GmbH (St. Leon-Rot), Bio-Rad-Laboratories (München) and MWG-Biotech AG (Ebersberg).

### *1.1 Acyllactyl-CoA and acylacryl-CoA synthesis*

Phenyllactyl-CoA, indolelactyl-CoA, p-hydroxyphenyllactyl-CoA and 2-hydroxyisocaproyl-CoA are synthesized following the modified method of Kawaguchi [24]. 50 µmol (*R*)-2-hydroxy acid und 60 µmol carbonyldiimidazole (9.73 mg) were added into 0.5 ml dry acetonitrile and mixed. The mixture was kept at room temperature for 2 minutes, then 50 µmol free coenzymeA dilithium salt (47.11 mg) in 0.5 ml 0.5 M $NaHCO_3$ was slowly dropped into the mixture. After incubation for 30 minutes the reaction was stopped by 1 M HCl to pH 2 and directly loaded on a 2 ml C18 Sep-PakTM column (Waters, USA), which was previously washed by 2 column volumes (CV) methanol and equilibrated by 5 CV 0.1% trifluoroacetic acid (TFA). After loading the column was washed with 5 CV 0.1% TFA and the CoA-ester was eluted by 0.1% TFA containing 50% acetonitrile. The elution fractions were lyophilized overnight and the mass of the synthesized CoA-ester was confirmed by MALDI-TOF mass spectrometry at the MPI for Terrestrial Microbiology, Marburg.

### *1.2 Instruments, gases and columns*

The Äkta FPLC system and the UV/vis spectrophotometer (Ultrospec 1100 pro and 2100 pro) were obtained from Amersham Biosciences (Freiburg). Quartz cuvettes were used for measuring UV/vis spectra and enzyme assay measurements below 320 nm. Disposable plastic cuvettes were used for measurements above 320 nm. All of which had a path length of 1 cm or 0.5 cm and a volume of 1 ml or 0.5 ml. Beckman (München) supplied the ultra-centrifuge, Sorval (München) the cooling centrifuges.

The column HiLoadTM26/60 SuperdexTM200 prep (gel filtration column) and the Sepharose chromatography media, DEAE (diethylaminoethyl) Sepharose Fast Flow (weak anion exchange

interaction), Q-Sepharose Fast Flow (strong anion exchange interaction) and Phenyl Sepharose 6 Fast Flow (High Sub) (hydrophobic interaction) for column packing were obtained from GE Healthcare Life Sciences (Sweden). The Strep-Tag II column was purchased from IBA GmbH (Göttingen).

### *1.3 Anaerobic work*

Anoxic experiments were done in a glove box (Coy Laboratories, Ann Arbor MI, USA) providing an atmosphere of $N_2/H_2$ (95% / 5%). Buffers for enzyme purification and enzyme assays were prepared by boiling and cooling under vacuum. Then the buffers were flushed with nitrogen and transferred to the anaerobic chamber and stirred overnight. Enzyme activity was determined inside the anaerobic chamber with Ultrospec 1100 *pro*.

## *2. Microbiological methods*

### *2.1 Bacterial strains and cultures*

*Clostridium sporogenes* (DSMZ 795 or ATCC 3584) stain was delivered freeze dried by the Leibniz-Institut, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). *C. sporogenes* was cultivated anaerobically in 100 ml serum bottles under a nitrogen atmosphere at 37 °C for ca. 18 h. One of the serum bottles was used to inoculate a 10 l pre-culture overnight for the 100 l fermenter culture of the organism.

Medium composition for 1 l culture medium:

| | |
|---|---|
| L-Phenylalanine | 40 mM (6.7 g) |
| or L-tryptophan | 40 mM (10.6 g) |
| Yeast extract | 5 g |
| Sodium thioglycolate | 1 g |
| Potassium phosphate buffer (1 M, pH 7.2) | 10 ml |
| Biotin | 1 mg |
| Trace-element solution (SL 10) | 1 ml |

Composition of the trace-element solution (SL 10) (Imhoff-Stuckle, 1983)

| | |
|---|---|
| HCl | 70 mM |
| $FeCl_2$ | 7.5 mM |
| $ZnCl_2$ | 0.5 mM |
| $MnCl_2$ | 0.5 mM |
| $H_3BO_3$ | 0.1 mM |
| $CoCl_2$ | 0.7 mM |
| $CuCl_2$ | 0.01 mM |
| $NiCl_2$ | 0.1 mM |
| $Na_2MoO_4$ | 0.15 mM |

*Escherichia coli* BL 21 and DH5α were cultivated in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl) containing antibiotic at 37 °C overnight. The strain DH5α [$F^-$ endA1 glnV44 thi-1 recA1 gyrA96 deoR nupG φ80d*lac*ZΔM15 Δ(*lacZYA-argF*)U169, hsdR17 ($r_K^-$ $m_K^+$), λ-] was used for gene cloning and BL21 (DE3) {F– ompT gal dcm lon hsdSB(rB- mB-) λ [DE3 (lacI lacUV5-T7 gene 1 ind1 sam7 nin5)]} for the gene expressions.

## *2.2 Plasmid*

The expression plasmid pASK-IBA7plus (tet-promoter, N-terminal Strep-Taction affinity tag Strep-tag II, cytosolic localization of the recombinant protein, $Amp^r$, Fig. 13) was used for the expression of the two genes encoding the dehydratase.

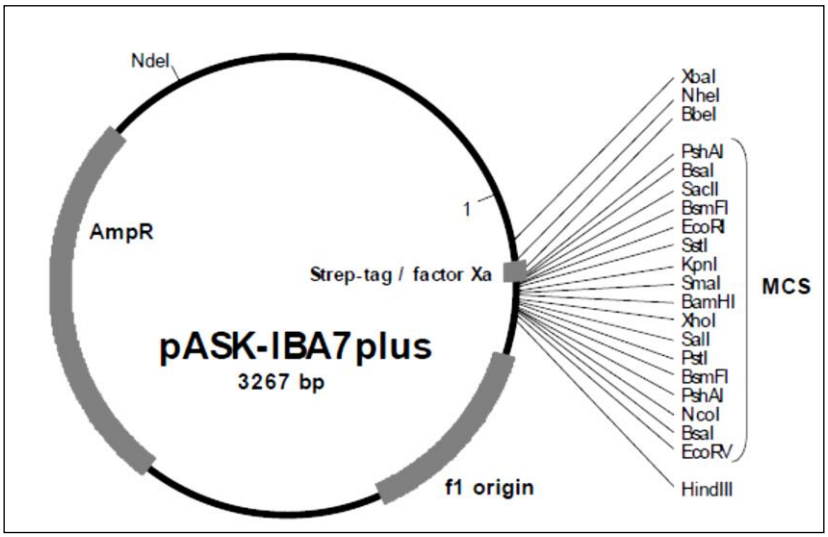



**Fig. 13** The vector map of pASK-IBA7plus

## *2.3 Antibiotics*

The antibiotics stock solution was prepared and used as described below in Table 3. All solutions of antibiotics were filtrated (0.3 µm).

**Table 3**

| Antibiotic | Stock in $H_2O$ | Final concentration in media |
|---|---|---|
| Carbenicilin | 200 mg/ml | 100 µg/ml |
| Ampicilin | 200 mg/ml | 100µg/ml |

## *2.3 Genomic DNA isolation from C. sporogenes*

Genomic DNA from *C. sporogenes* was isolated from 1 g wet cells. Cells were suspended in 3 ml Tris-sucrose buffer (10 mM Tris/HCl pH 8.0, 25% sucrose) and incubated at 37 °C for 90 min with gentle shaking after adding 100 mg lysozyme. Then 4 ml 10 mM Tris/HCl pH 8.0 and 25 mM EDTA was added and incubated on ice for 15 min. After adding 20 mg proteinase K and 100 mg RNase the solution was incubated at 37 °C for 3 h. For extraction 10 ml phenol/chloroform (1:1) was added in the mixture and shaken gently. The aqueous and organic phase were separated by centrifugation at 5,000 × *g* for 20 min, this step was repeated three times. The aqueous phase containing the nucleic acid was extracted with an equal volume of chloroform/isoamylalcohol (24:1) and the protein was removed by centrifugation at 5,000 x g for 10 min. The aqueous phase was transferred to a dialysis bag for overnight dialysis in TE buffer (10 mM Tris/HCl, 1mM EDTA, pH 8.0) at 4 °C.

## *2.4 Plasmid DNA isolation*

Plasmid DNA isolation was done by alkaline lysis methods using GeneJET™ Plasmid Miniprep Kit (Fermentas). LB medium (5 ml) containing antibiotics was inoculated with one single bacterial colony and incubated overnight at 37 °C. The culture was transferred into an Eppendorf tube and harvested at 13,000 × *g* in a microcentrifuge for 2 minutes. The bacterial pellet was suspended in 250 µl Solution I (50 mM glucose, 10 mM EDTA, 25 mM Tris/HCl pH 8.0), and then lysed in 25 µl Solution II (0.2 M NaOH. 1% SDS) and neutralized with 300 µl Solution III (3 M potassium acetate/glacial acetic acid, pH 4.8). In the end the soluble supernatant was separated from cell debris by centrifugation for 5 minutes and transferred into a filter connected to a new Eppendorf tube. The plasmid DNA was washed two times with wash buffer and eluted with TE buffer (10 mM Tris/HCl pH8.0, 1 mM EDTA).

## *2.5 Agarose gel electrophoresis*

Agarose powder (1.8 or 2.0 % w/w) was mixed with TAE buffer (40 mM Tris/acetate, 1 mM EDTA) and heated in microwave oven until it completely melted and boiled. After cooling the agarose solution to about 60 °C, it was poured into a casting stray containing a sample comb and allowed to solidify at room temperature. The gel was inserted horizontally into the electrophoresis chamber just covered with TAE buffer. DNA samples mixed with 6 x MassRuler™ Loading Dye solution (10 mM Tris/HCl pH 7.6, 0.03% bromophoenol blue, 60% glycerol and 60 Mm EDTA) were then pipetted into the sample wells. Bromophenol blue dye migrates through the agarose gel at the front of DNA fragments. The gel was stained with ethidium bromide and placed on an ultraviolet transilluminator.

## *2.6 DNA extraction from agarose gels*

DNA bands on the agarose gel were exposed on an UV-illuminator using a short wavelength and rapidly cut from the gel. Extraction was performed following the manual of the QIAquick Gel Extraction Kit (QIAGEN GmbH).

## *2.7 DNA restriction and ligation*

Restriction reactions were performed following the enzyme insert manual. T4-DNA ligase (Fermentas GmbH) was used for ligations of double stranded DNA.

## *2.8 Dialysis of ligation mixtures*

After ligation the mixture was dialyzed before electro transformation. The ligation mixture was pipetted on a Millipore-Membrane (#VSPWP 02500) which was floating on water. After 30-60 minutes of dialysis, the ligation mixture was carefully recovered from the membrane.

## *2.9 Electrotransformation*

The dialyzed ligation mixture was added to 40 µl electro-competent cells and transferred to a Gene-Pulser cuvette (Bio-Rad). A pulse was given to the cuvette using the following settings: 25 µF, 1.8 kV and 200 Ohm. The cuvette was washed with 300 µl LB medium and transferred to a sterile 1.5 ml Eppendorf tube. The transformation mixture was incubated for 30 minutes at 37 °C and then plated on a LB agar plate containing antibiotic(s). The agar plate was incubated overnight at 37 °C to get colonies.

## *2.10 Determination of DNA concentration and purity*

The DNA concentration and purity were determined measuring $OD_{260}$ and $OD_{280}$:

OD260 = 1 corresponds to 50 µg/ml of dsDNA

OD260/OD280 < 1.8 indicates contamination with protein or phenol

OD260/OD280 > 1.8 indicates contamination with RNA

OD260/OD280 ≈ 1.8 indicates pure dsDNA

## *2.11 Preparation of competent E. coli cells for electro transformation*

A fresh single *E. coli* colony from a plate was inoculated in 5 ml LB medium and incubated overnight. The 5 ml culture was inoculated into a 500 ml main culture and grown until the exponential phase (OD600 = 0.5 – 0.8). The cells were harvested by a pre-cooled (4 °C) high-speed centrifuge with 6000 x g for 20 minutes. The washed cells were suspended with 1 ml 10% glycerol and 40 µl aliquots in thin-wall 500 µl tubes were stored at − 80 °C.

## *2.12 PCR primers*

Restriction enzyme KpnI cleavage site in the forward primer:

5'...G G T A C^C...3'
3'...C^C A T G G...5'

Forward primer: ACTGCGGTACC ATG AGT GAT AGA AAT AAG GAA GTA AAA GAA AAA AAG GCA

Restriction enzyme NcoI cleavage site in the reverse primer:

5'...C^C A T G  G...3'

3'...G  G T A C^C...5'

Reverse primer: ATGCGCCATGG TTAA AGT GTT TCT GAA AAA GCC TGA ATT GCA GT

## *2.13 PCR reactions*

The PCR reactions were performed using Phusion polymerase (Finnzymes and BioLabs), which is a high fidelity DNA polymerase. The reaction mixtures were made with following concentrations and cycling program:

Concentration of ingredients

| | Final concentration |
|---|---|
| 5 * HF-Buffer mixer | |
| dNTPs | 200 µM |
| Template DNA | 1~ 2 ng/µl |
| Forward primer | 1 pM |
| Reverse primer | 1 pM |

Cycling program:

| | | |
|---|---|---|
| 1. | 98 °C | 30 s |
| 2. | 98°C | 30 s |
| 3. | 65°C | 30 s |
| 4. | 72 °C | 69 s |
| 5. | 72°C | 10 min |

30 cycles from 2 to 4

### *2.14 Cloning of the genes*

The DNA fragment of the gene encoding aryllactyl-CoA dehydratase *fldBC* (2350 bp) was amplified with the designed primers containing restriction cut sites depending on the multicloning site of the pASK-IBA7plus vector.

### *2.15 Sequencing of the cloned genes*

Primers described below were used to check sequence of the cloned genes.

Primers for pASK-IBA vectors

pASK-IBA forward primer: 5'-AGA GTT ATT TTA CCA CTC CCT-3'

pASK-IBA reverse primer: 5'-GCT CCA TCC TTC ATT ATA GC-3'

## *3. Biochemical methods*

### *3.1 End product and intermediates determination via ESI-Mass spectrometry and NMR*

The end product and intermediates from the growing cultures on phenylalanine and tryptophan were determined via ESI-MS (electro spray ionization mass spectrometry). The ESI-MS was performed on a Finnigan LTQ-FT (Thermo Fisher Scientific, Germany) mass spectrometer at Department of Chemistry of the Philipps Universtiy Marburg. The samples were dissolved in methanol and subjected to ESI-MS in the negative ion mode.

For the determination of intermediates an inactivator 2,4-dinitrophenol (DNP) was added to the growing culture during the exponential phase with $OD_{598}$ = 0.3. After further 10-14 hours the cells were removed by centrifugation. The supernatants were mixed with conc. HCl or $H_3PO_4$ to pH 2.0 and extracted 3 times with 100 ml ethyl acetate. The organic phases were first dried with $Na_2SO_4$, after filtration the organic phases were concentrated by Rotary evaporator to brown oils.

### *3.2 Protein Purification*

#### *3.2.1 Methods of cell disruption*

For the native and recombinant protein purification two methods were used to disrupt cells. The disruption was carried out under anoxic condition in a glove-box.

**Ultrasonic disintegration (Sonication)**

Cells were suspended in the appropriate buffers and filled into a glass Rosetta cell, kept on ice-water and broken by ultrasonication on a Branson 250 Sonifier (Heinemann, Germany). The duty time was 3-5 minutes at 60% duty cycle. The process was repeated several times.

**French Press**

The cell suspension was sucked into a pre cooled French press cell (American Instruments, Maryland, USA) and the cells were disrupted by applying a pressure of 110 MPa. The broken cells were collected into falcon tube and refilled into the pressure cell for another cycle of disrupting. The cycle was repeated 3-4 times.

### *3.2.2 Native protein purification of aryllactyl-CoA dehydratase from C. sporogenes grown on tryptophan or phenylalanine*

All steps of native protein purifications were performed in an anaerobic glove box.

The native aryllactyl-CoA dehydratases were purified from *C. sporogenes* grown on phenylalanine or tryptophan, but followed the same protocol as shown below.

About 20 g cells of *C. sporogenes* were suspended in 40 ml 20 mM Mops buffer pH 7.5, which contained 2 mM dithiothreitol (DTT), 2 mM dithionite. The cells were disrupted via ultrasonication as described before and centrifuged at 100,000 × *g* for 1 hour at 4 °C. The supernatant was filtrated via a membrane (0.45 µm) and loaded on a DEAE-Sepharose column, which was equilibrated with 3 column volumes of 20 mM Mops pH 7.3 containing 2 mM DTT. The elution was performed by running a linear gradient from 0 M to 0.6 M NaCl in 20 mM Mops buffer in 7 column volumes. The pooled fractions were collected and concentrated with an Amicon-concentrator (30 kDa pore size, Witten, Germany).

After concentration the protein solution was mixed with equal volume of 2 M $(NH_4)_2SO_4$ to get the final concentration of 1M $(NH_4)_2SO_4$ and loaded on a Phenyl-Sepharose column equilibrated with 3 column volumes of 20 mM Mops pH 7.3 containing 1 M $(NH_4)_2SO_4$ and 2 mM DTT. The

enzyme was eluted with a linear gradient of 1.0-0.3 M $(NH_4)_2SO_4$ in 20 mM Mops buffer in 7 column volumes.

The combined fractions containing dehydratase were desalted and concentrated via a Centricon-concentrator (VIVASPIN20, 50,000 MWCO PES, Sartorius, Germany) to 1-2 ml. The protein was further purified by chromatography on a Superdex$^{TM}$200 prep column in 20 mM Mops pH 7.3 and 150 mM NaCl.

### *3.2.3 Separation of CoA-transferase from the aryllactyl-CoA dehydatase complex*

After the Phenyl-Sepharose column, the purified dehydatase complex was desalted and transferred to a Q-Sepharose column equilibrated with 20 mM Mops pH 7.3 containing 2 mM DTT. A linear gradient from 0 to 0.8 M NaCl in 20 mM Mops pH 7.3 was performed in 8 column volumes.

### *3.2.4 Gene expression and purification of FldBC*

The plasmid pASK-IBA7*plus* harboring genes encoding the two subunits of aryllactyl-CoA dehydratase *fldBC* was transformed into *E. coli* BL21 (DE). An overnight 100 ml preculture inoculated with a fresh single colony from LB-agar plate was used to inoculate 1 l Standard I-medium containing carbenicillin (100 µg/ml) at 37 °C or room temperature. When the 1 l culture reached the middle of the exponential phase with $OD_{598}$ = 0.6-0.7 gene expression was induced with anhydrotetracycline (AHT, 200 µg/l). Cells were harvested after 5 hours or overnight and washed with anaerobic buffer under anoxic conditions. The cells were lysed by French press or sonication. Cell debris was removed by ultracentrifugation at 100,000 × *g* at 4 °C for 60 min.

The filtrated supernatant was directly applied on a StrepTactin column, which previously was equilibrated with buffer A (20 mM Mops pH 7.3, 150 mM NaCl and 2 mM DTT). After loading the supernatant, the column was washed with 5 column volumes of buffer A. The protein was eluted with buffer E (buffer A containing 3 mM desthiobiotin). The elution fractions containing dehydratase were collected, concentrated by Centricon and stored at – 80 °C.

### 3.2.5 *Purification of the recombinant activator HgdC of 2-hydroxyglutaryl-CoA dehydratase of* Acidaminococcus fermentans

Cells of *E. coli*, which harbored the plasmid pASK-IBA3(+) with the gene *hgdC* from *A. fermentans*, were suspended in buffer A (50 mM Mops pH 7.4, 300 mM NaCl, 10 mM $MgCl_2$ and 5 mM DTT) and disrupted by a French press. After centrifugation for 1 hour at 100,000 × *g* at 4 °C the cell-free extract was applied on a StrepTactin column equilibrated with buffer A. Elution was performed with buffer E (buffer A with 3 mM desthiobiotin and 2 mM ADP). The fractions containing enzyme were collected and concentrated via Centricon concentrator (30,000 kDa cut off).

### 3.2.6 *Preparation of* C. sporogenes *membranes*

About 2 g wet packed *C. sporogenes* cells of were suspended in 4 ml 50 mM Kpp buffer pH 7.4 and broken by three passages through a mini French press at 110 MPa in an anaerobic chamber. Cell debris was removed by centrifugation at 10, 000 × *g* for 20 minutes at 4 °C. The crude extract was centrifuged at 120,000 × *g* for 1 hour. The membrane extract was collected and washed twice with 50 mM KPP pH 7.4 by centrifugation at 120,000 × *g* for 30 minutes. The washed membrane extract was solubilized with 40 mM n-dodecyl-β-D-maltoside (DM) and homogenized well to solubilize it further. The suspension was centrifuged at 120,000 × *g* for 30 minutes. The supernatant was used to determine Rnf. [25]

## 3.3 *Determination of the protein concentration*

**Bradford method**

Protein concentrations were determined by the Bradford method (Bradford, 1976). The method is based on the principle that the absorption maximum for an acidic solution of Coomassie Brilliant Blue shifts from 465 nm to 595 nm upon binding to protein. 0-10 µg BSA as standers were made up to an 800 µl volume with water and 200 µl Coomassie Brilliant Blue G-250 reagent (the Biorad-Microassay reagent 1:5 with water). The reactions were incubated in the dark at room temperature for 10 minutes and the absorption was measured at 595 nm.

**Absorbance of protein from the primary sequence:**

The molar extinction coefficient of a particular protein in pure form can be calculated from the primary sequence. It based on the absorption of aromatic amino acids tyrosine and especially tryptophan. The sequences of the protein samples were analyzed and the molar extinction coefficients were calculated by the online tool of EnCor (Biotechnology Inc., Florida, USA). The samples were scanned in quartz cuvettes by a UV/vis spectrophotometer. The absorbance at 280 nm was used to calculate the protein concentrations.

## *3.4 Polyacrylamide gel electrophoresis (PAGE)*

**Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)**

The protein samples were mixed with SDS sample buffer (125 mM Tris/HCl, pH 6.8, 10% glycerol, 10% mercaptoethanol, 4% SDS, 0.2% bromophenol blue) in the ration of 1:1 and boiled at 95 °C for 10 minutes to denature the proteins. The running buffer was 25 mM Tris pH 8.8, 190 mM glycine, 0.1% of SDS. Electrophoresis was run at constant voltage of 200 mV until the bromophenolblue marker reached the end of the gel. The proteins on the gel were stained with 0.1% Coomassie Brilliant Blue R-250 in methanol/water/glacial acetic acid (4:5:1) overnight or shortly by heating in a microwave. The gel was destained by a solution of ethanol/water/glacial acetic acid (4:5:1) overnight on a shaker.

**Table 4** SDS-PAGE gel content

| Stock solutions | Separating gel 8% (µl) | Stacking gel 12% (µl) |
|---|---|---|
| 1 M Tris / HCl pH 8.8 | - | 4500 |
| 1 M Tris / HCl pH 6.8 | 470 | - |
| $H_2O$ | 2540 | 2080 |
| 10 % SDS | 40 | 120 |
| Acrylamid/Bisacrylamid (30%) | 950 | 5000 |
| 5 % TEMED | 40 | 120 |
| 10 % Ammoniumperoxodisulfat (APS) | 80 | 180 |

**Native PAGE**

For molecular mass determination of native enzymes the gel was prepared following the content in Table 5. Bovine serum albumin (BSA) was used as protein marker. The protein samples were mixed with sample buffer in a ratio of 1:1. The running buffer was 25 mM Tris, 192 mM glycine, pH 8.3. Electrophoresis was run at 8 mA at 4 °C until the blue color reached the end of the gel. Staining and destaining steps were as same as SDS-PAGE.

**Table 5** Native PAGE 8%

| Stock solutions | 8% (µl) |
|---|---|
| 1.5 M Tris / HCl pH 8.8 | 375 |
| $H_2O$ | 720 |
| Acrylamid/Bisacrylamid (30%) | 400 |
| 5% TEMED | 950 |
| 10% Ammoniumperoxodisulfat (APS) | 50 |

## *3.5 Iron determination*

**Non-heme iron determination with Ferene:**

The non-heme iron in proteins is liberated by treatment with hydrochloric acid. After neutralization with ammonium acetate, $Fe^{3+}$ is converted to $Fe^{2+}$ by reduction with ascorbic acid. Protein is precipitated and dissolved by sodium dodecylsulphate (SDS). The iron chelator Ferene, 3-(2-pyridyl-)-5,6-bis(5-sulfo-2-furyl)-1,2,4-triazine, disodium salt trihydrate, forms with iron a blue complex. [A. Pierik, Analysis of metals and acid-labile sulphide in proteins, EU practical training course manual]

Reagents:

| | |
|---|---|
| HCl | 1% (w/v) |
| Ammonium acetate solution | 7.5% (w/v) |
| SDS | 2.5% (w/v) |
| Ascorbic acid | 4.0% (w/v) |
| Ferene | 1.5% (w/v) |

For the calibration curve a freshly prepared solution of 0.2 mM $(NH_4)Fe(SO_4)_2$ x 6 $H_2O$ (Mohr's salt) was used. Six standard samples of the Mohr's salt solution (10, 20, 40, 80, 100 µl), 100 µl $H_2O$ as blank and 2 diluted protein samples were filled with $H_2O$ to an end volume of 100 µl. The samples were mixed with 1 % HCl and incubated at 80 °C for 10 minutes. After cooling down to room temperature 500 µl ammonium acetate solution, 100 µl ascorbic acid, 100 µl SDS and 100 µl Ferene were sequentially added and vortexed after each addition. The tubes were centrifuged at 13000 × *g* for 10 minutes. The absorbance of the supernatants was measured at 593 nm against $H_2O$. The iron content of the protein was calculated from the calibration curve.

**Iron-Sulfur- Cluster determination**

Protein containing [4Fe-4S] clusters in the reduced state displays a shoulder around 400 nm in UV/vis spectrum with an extinction coefficient of 16 $mM^{-1}cm^{-1}$ at 400 nm and 14.5 $mM^{-1}cm^{-1}$ at 385 nm per iron atom. Together with the typical absorbance extinction coefficient of aryllactyl-CoA dehydratase at 280 nm (3.5.3) the content of [4Fe-4S]-cluster was calculated from UV/Vis wavelength spectrum of protein samples.

## *3.6 MALDI-TOF mass spectrometry*

The synthesized CoA compounds were confirmed and identified by MALDI-TOF (Matrix-assisted laser desorption/ionization time of flight) mass spectrometry. The matrix was alpha-cyano-4-hydroxy cinnamic acid (CHCA, Sigma) dissolved in 70% acetonitrile / 0.1% TFA (trifluoro acetic acid). 1 µl sample was mixed with 1 µl CHCA and spotted onto a gold plate in a dilution series. The measurements were performed with a 355 nm laser in positive reflector mode with a delayed extraction with a positive polarity on the Proteomics Analyzer 4800 mass spectrometer (Applied Biosystems, MDS Sciex, Framingham, USA) at Max Planck-Institute for Terrestrial Microbiology, Marburg.

The peptide sequences of protein samples from SDS-PAGE were also analyzed by MALDI-TOF MS. The protein bands were cut out from the gels and distained with 30% isopropanol containing 20 mM $NH_4HCO_3$. After distaining, the gel pieces were dehydrated with 100% isopropanol and dried. Then the pieces were redehydrated in 5 mM $NH_4HCO_3$ in 10% acetonitrile containing 0.013 g/l sequencing-grade modified protease trypsin and incubated overnight at room temperature. The digested pepetides were analyzed by Nano-LCMS using the Proteomics Analyzer 4800 mass spectrometer.

## *3.7 Enzyme activity assays*

All enzyme activity assays were performed on Ultrospec 1100 *pro* spectrophotometer using 0.5 ml Quartz cuvettes. The Michaelis-Menten parameters were calculated via GraphPad Prism software.

### *3.7.1 Aryllactyl-CoA dehydratase*

**Assay components:**

| | |
|---|---|
| Tris/HCl pH 8.0 | 50 mM |
| $MgCl_2$ | 2.5 mM |
| ATP | 0.4 mM |
| Dithionite | 0.1 mM |
| Activator HgdC | 2 μg/ml |
| Aryllactyl-CoA | 20 μM |
| Dehydratase | |

**Reaction:** Aryllactyl-CoA → Arylacrylyl-CoA + $H_2O$

Aryllactyl-CoA dehydratase activity was measured under anoxic conditions at room temperature. The assay components except substrates were mixed and incubated for 30 minutes at room temperature. The reactions were started by adding the synthesized aryllctyl-CoA (indolelactyl-CoA, phenyllactyl-CoA and 2-hydroxyisocaproyl-CoA). The formation of (*E*)-indoleacrylyl-CoA was followed at 380 nm ($\varepsilon_{380}$ = 6.5 $mM^{-1}cm^{-1}$), cinnamoyl-CoA at 310 nm ($\varepsilon_{310}$ = 2.26 $mM^{-1}cm^{-1}$), 2-isocaprenoyl-CoA at 290 nm ($\varepsilon_{290}$ = 2.2 $mM^{-1}cm^{-1}$).

### *3.7.2 CoA-transferase*

The activity of CoA-transferase of aryllactyl-CoA dehydratase complex was measured aerobically in a mixer of 50 mM potassium phosphate pH 7.0, 20 μM (*R*)-indolelactate and 10 μM (*E*)-indoleacrylyl-CoA. The reaction was initiated with adding of enzyme and followed by the decrease in absorbance at 380 nm ($\varepsilon_{380}$ = 6.5 $mM^{-1}cm^{-1}$).

### *3.7.3 Tryptophan transaminase*

**Assay components:**

| | |
|---|---|
| Tris/HCl pH 8.4 | 0.5 mM |
| Pyridoxal-5-phoshate (PLP) | 0.5 μM |
| α-Ketoglutarate | 12 μM |
| Tryptophan | 20 μM |

Enzyme in cell-free extract

The activity of tryptophan transaminase in cell-free extract was measured by following the absorption change of the formation of the enol of indolepyruvate at 305 nm ($\varepsilon_{305}$ = 3.9 $mM^{-1}cm^{-1}$). PLP, α-ketoglutarate and cell-free extract were incubated in Tris/HCl pH 8.4 buffer for 5 minutes. The reaction was started with addition of tryptophan. [26]

### *3.7.4 Indoleacrylate reductase*

**Assay components:**

| | |
|---|---|
| Tris/HCl pH 8.0 | 50 mM |
| Dithionite | 0.1 mM |
| NADH | 0.1 mM |
| Indoleacrylate | 0.5 mM |

Enzyme

**Reaction:** Indoleacrylate + NADH + $H^+$ → Indolepropionate + $NAD^+$

The enzyme activity assays were performed anaerobiclly at room temperature with cell-free extract or partial purified indoleacrylate reductase. NADH and indoleacrylate were mixed in Tris/HCl buffer; the reactions were started with adding of enzyme and followed the decrease of absorbance of NADH at 340 nm ($\varepsilon_{340}$ = 6.2 $mM^{-1}cm^{-1}$).

### *3.7.5 Indolelactate dehydrogenase*

**Assay components:**

| | |
|---|---|
| Tris/HCl pH 8.0 | 50 mM |
| Dithionite | 0.1 mM |
| NADH | 0.1 mM |
| Indolepyruvate | 0.8 mM |
| Enzyme | |

**Reaction:** Indolepyruvate + NADH + $H^+ \rightarrow$ Indolelactate + $NAD^+$

The enzyme activity assays were performed aerobiclly at room temperature with cell-free extract or partial purified indolepyruvate dehydrogenase. NADH and enzyme were mixed in Tris/HCl buffer; the reactions were started with adding of indoleacrylate and followed the decrease of absorbance of NADH at 340 nm ($\varepsilon_{340}$ = 6.2 $mM^{-1}cm^{-1}$).

### *3.7.6 Rnf with ferricyanide*

**Reaction:**

$$NADH + 2[Fe(CN)_6]^{3-} \xrightarrow{\text{Rnf complex in CE}} NAD^+ + 2[Fe(CN)_6]^{4-} + H^+$$

The activity of Rnf complex in cell-free extract was measured anaerobically in 50 mM Kpp pH 7.4 containing 0.2 mM NADH and 2 mM ferricyanide in a total volume of 0.5 ml at room temperature. The reaction was started by adding enzyme and followed by the decrease of ferricyanide absorbance at 420 nm ($\varepsilon_{420}$ = 1.02 $mM^{-1}cm^{-1}$). [25]

# *RESULTS*

## *1. Fermentations of phenylalanine and tryptophan in* Clostridum sporogenes

### *1.1 Growth of C. sporogenes on tryptophan or phenylalanine*

The first step of the investigation of the fermentation of tryptophan in *C. sporogenes* was to grow this organism in a yeast extract medium to check and find out the optimal concentration of tryptophan. At the same time the growth on tryptophan was also compared with that on phenylalanine. The growth curves of the media with 40 mM, 60 mM, 80 mM and 100 mM tryptophan or phenylalanine are shown in Fig. 14 a-d. Media with 20 mM substrates exhibited almost the same growth as the reference medium containing only yeast extract. The media with 100 mM and 120 mM tryptophan or phenylalanine were already over saturated and the amino acids crystallized out. Generally the bacteria grew better in phenylalanine medium than in tryptophan medium at all concentrations of between 40 mM and 80 mM; furthermore phenylalanine dissolves in water better than tryptophan. The final OD value of growth on tryptophan was just around 0.45 and that on phenylalanine 0.65. Later it was observed that with 1% peptone or tryptone the bacteria grew much better with a final OD of 0.9. Therefore in the subsequent work a mixed medium with 1% peptone, 0.5% yeast extract and 40 mM tryptophan or phenylalanine was used to grow *C. sporogenes.* Without added amino acids OD of 0.12 was reached.

The bacterium was grown anaerobically on tryptophan or phenylalanine media at 37 °C for 16 hours in a 100 l fermenter. The cells were stored at -80 °C.

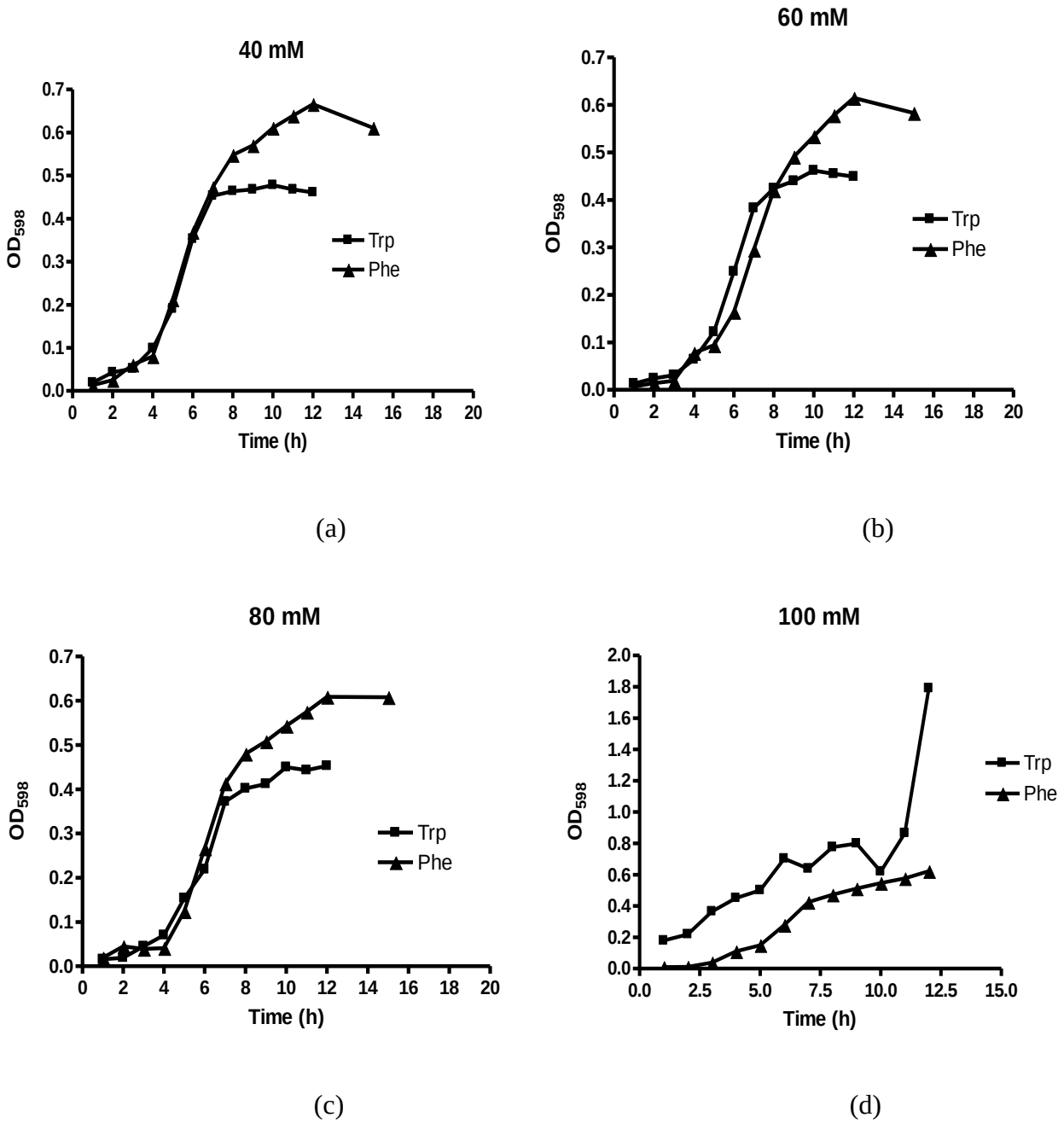



**Fig.14** Growth curves of *C. sporogenes* on media with tryptophan or phenylalanine

The growth experiments indicated that *C. sporogenes* is able to use tryptophan as energy and carbon source for growth, but the maximum OD value of growth was only 0.45. It is proposed that the fermentation of tryptophan by *C. sporogenes* should follow the Stickland reaction (Introduction 1.2). Tryptophan could be oxidized as well as reduced by *C. sporogenes*, this is to say, tryptophan is able to disproportionate. Peptone is rich in amino acids but almost no tryptophan. In contrast to the media containing only tryptophan and yeast extract, in the media with additional peptone the bacteria could grow to almost OD 1.0. Probably peptone contains a certain amino acid that could be more effectively oxidized and act as a better oxidation

partner to support the reduction of tryptophan. Therefore, the bacteria were grown in several tryptophan media with typical electron donor amino acids in Stickland reactions like alanine, valine and isoleucine. The results in Table 6 (a) and (b) show, however, that growth of *C. sporogenes* in the tryptophan media was not significantly stimulated by alanine, valine and isoleucine, though the fermentation of isoleucine as Stickland reaction partner by *C. sporogenes* has been investigated [11]. However, with casamino acids or especially peptone the bacteria grew much better. Later it will be shown that the oxidation partners of tryptophan could be arginine or proline.

**Table 6 (a)** Growth in 40 mM tryptophan (1%) media with alanine or peptone

| Growing on | after 18 h OD $_{(595\ nm)}$ |
|---|---|
| Tryptophan + Yeast (0.25%) | 0.45 |
| Tryptophan + Alanine (0.5%) + Yeast (0.25%) | 0.49 |
| Tryptophan + Alanine (1%) + Yeast (0.25%) | 0.51 |
| Tryptophan + Peptone (0.5%) + Yeast (0.25%) | 0.94 |

**(b)** Growth in 20 mM tryptophan (0.5%) media with different electron donors for the Stickland reaction

| Growth after | OD $_{(595\ nm)}$ 18 h | 24 h |
|---|---|---|
| Tryptophan + Yeast (0.2%) | 0.2 | 0.21 |
| Tryptophan + Yeast (0.2%) + Valine (10 mM) | 0.18 | 0.17 |
| Tryptophan + Yeast (0.2%) + Isoleucine (10 mM) | 0.17 | 0.16 |
| Tryptophan + Yeast (0.2%) + Casamino acid (1%) | 0.66 | 0.67 |

## *1.2 Intermediates and end products*

Based on the structural similarity between tryptophan and phenylalanine the pathway of the fermentation of tryptophan to indolepropionate in *C. sporogenes* is proposed to follow that of phenylalanine[8] via indolepyruvate, which is oxidized to indolelacetate and reduced via indolelactate and indoleacrylate to indolepropionate. To establish this pathway 2,4-dinitrophenol (DNP) was added as inhibitor to the culture containing 40 mM tryptophan and 0.5% yeast extract in the middle of the exponential growth phase. It has been observed that the nitro groups of DNP can take the high energy electron from the activator of the dehydratase or possibly also from the reductase [27, 28]. Thus addition of DNP caused inhibition of the fermentation. With different concentrations of DNP the dehydration and the reduction of lactate to propionate can be selectively inhibited. Whereas 20 µM DNP inhibited both reactions, with 10 µM DNP only the reduction was affected [27].

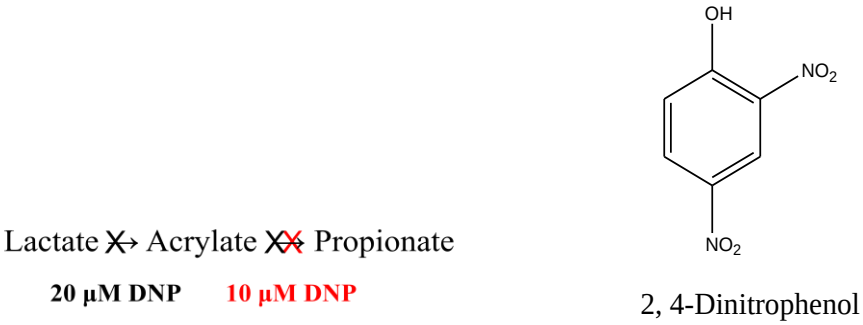



**Fig. 15** Inhibition effect of DNP on the fermentation of lactate

After addition of DNP to a growing culture of *C. sporogenes*, the bacteria grew much more slowly (Fig. 15). The extracted compounds from the growth supernatant were determined via HPLC and ESI-MS (Fig. 16a and b); the results are summarized and shown in Table 7. With 10 µM DNP indolelactate and indoleacrylate were found in the medium and with 20 µM only indolelactate was found. The end product indolepropionate was found in all media during the exponential phase, but indoleacetate was found in none of the media. This result matches with those obtained by Elsden on the end products of the metabolism of aromatic amino acids by clostridia [7].

**Table 7** Accumulation of intermediates in tryptophan-cultures (with 0.5% yeast extract) in the presence of 2, 4-dinitrophenol (DNP) as analyzed by mass spectrometry

| Compound (mass in Da) | + 10 μM DNP | + 20 μM DNP |
|---|---|---|
| Indolepropionate **(**188) | + | + |
| Indolelactate **(**204) | + | + |
| Indoleacrylate (189) | + | - |
| Indoleacetate **(**175) | - | - |

The bacterium was also grown in a tryptophan (1% w/v) medium and a phenylalanine (1% w/v) medium with reduced concentrations of yeast extract (0.25%). Unexpectedly indoleacetate was found in the tryptophan-medium, as well as phenylacetate in the phenylalanine-medium (Table 8).

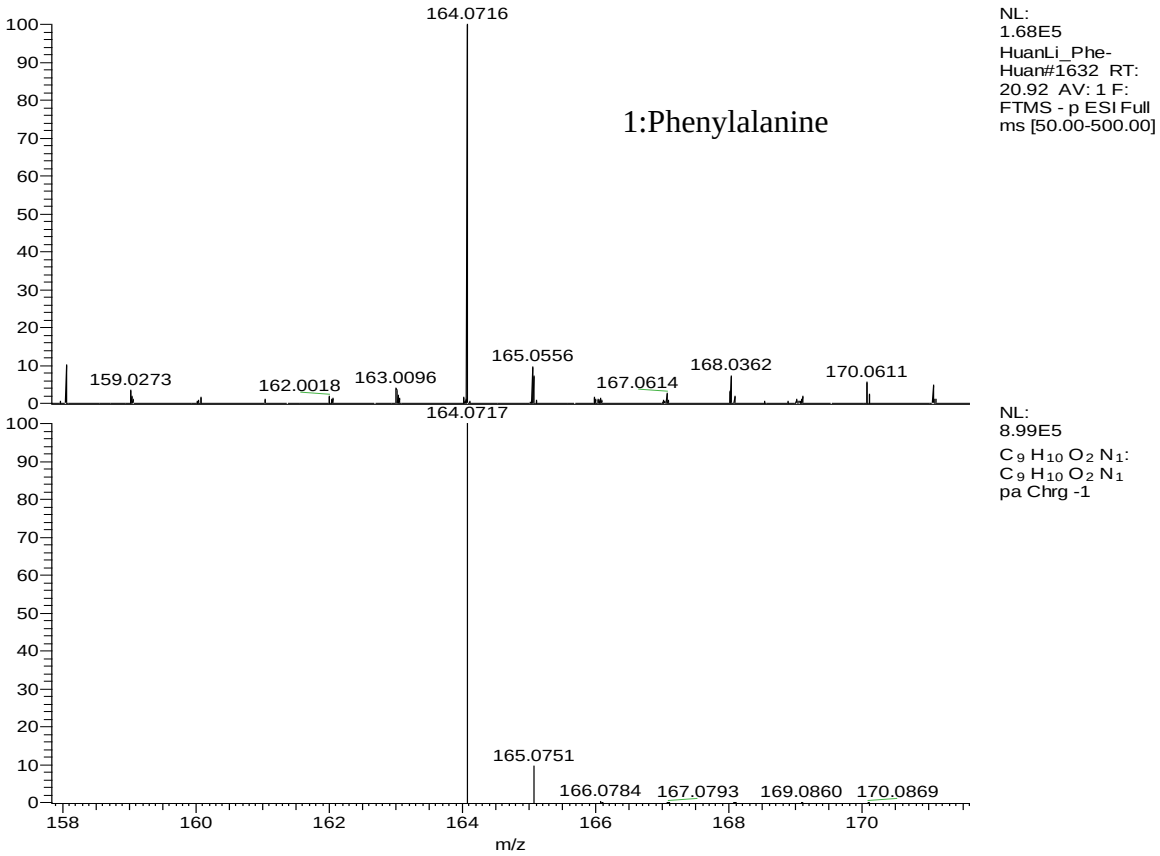
**Table 8** Determination of intermediates via ESI-MS

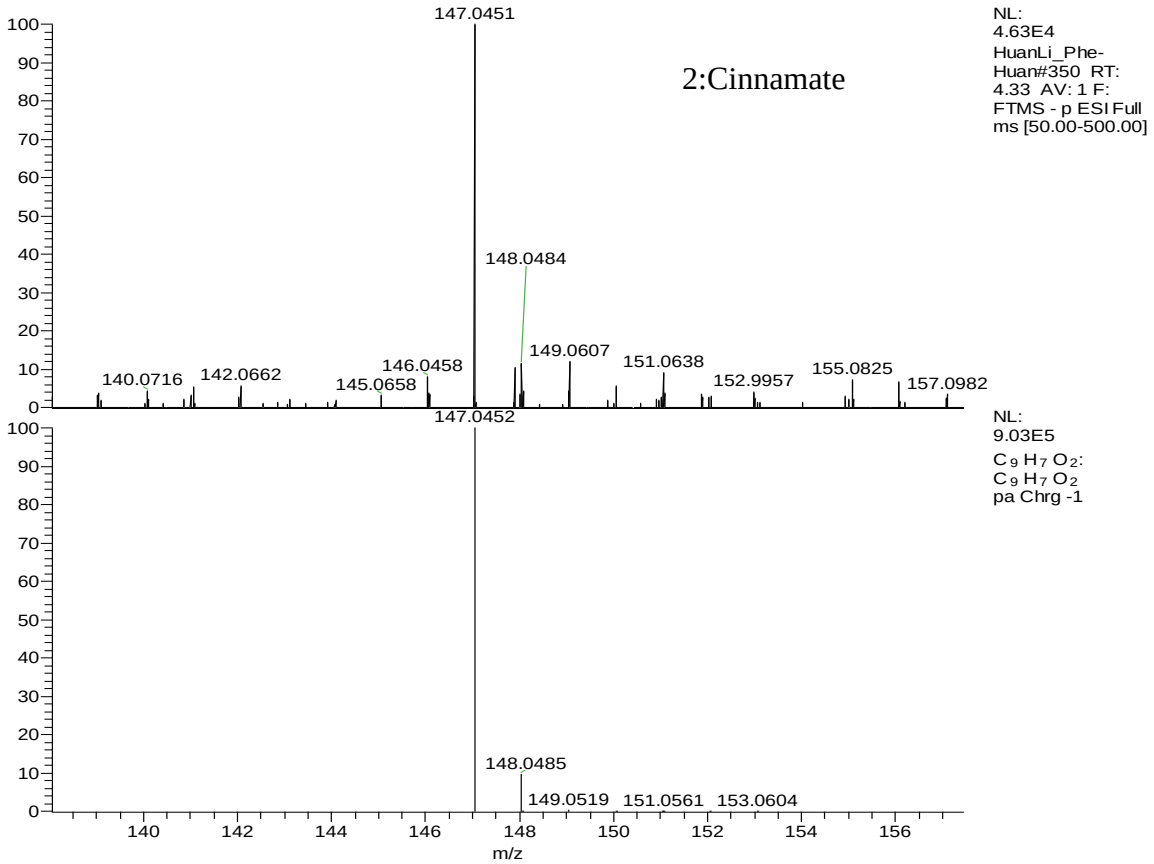
**(a)** 40 mM Tryptophan + Yeast extract

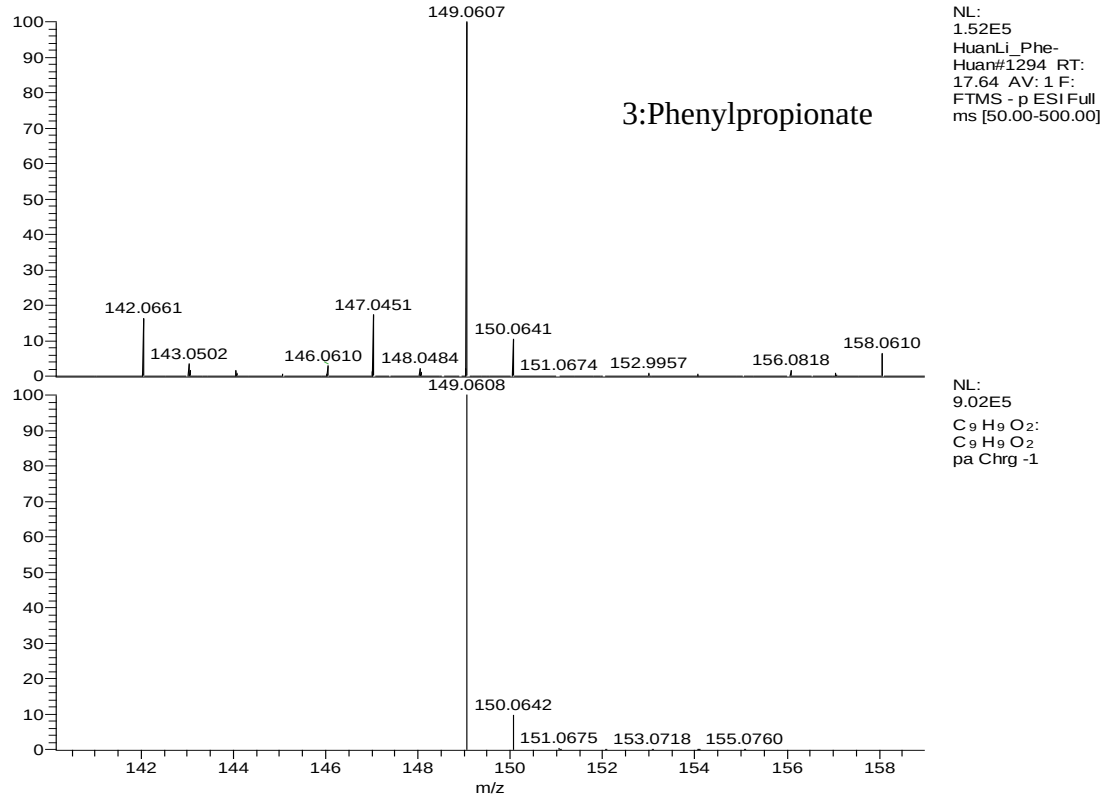
| | Indolelactate | Indoleacrylate | Indolepropionate | Indoleacetate |
|---|---|---|---|---|
| Tryptophan + Yeast extract (**0.25%**) | + | + | + | + |
| Tryptophan + Yeast exract (**0.5%**) | + | + | + | - |

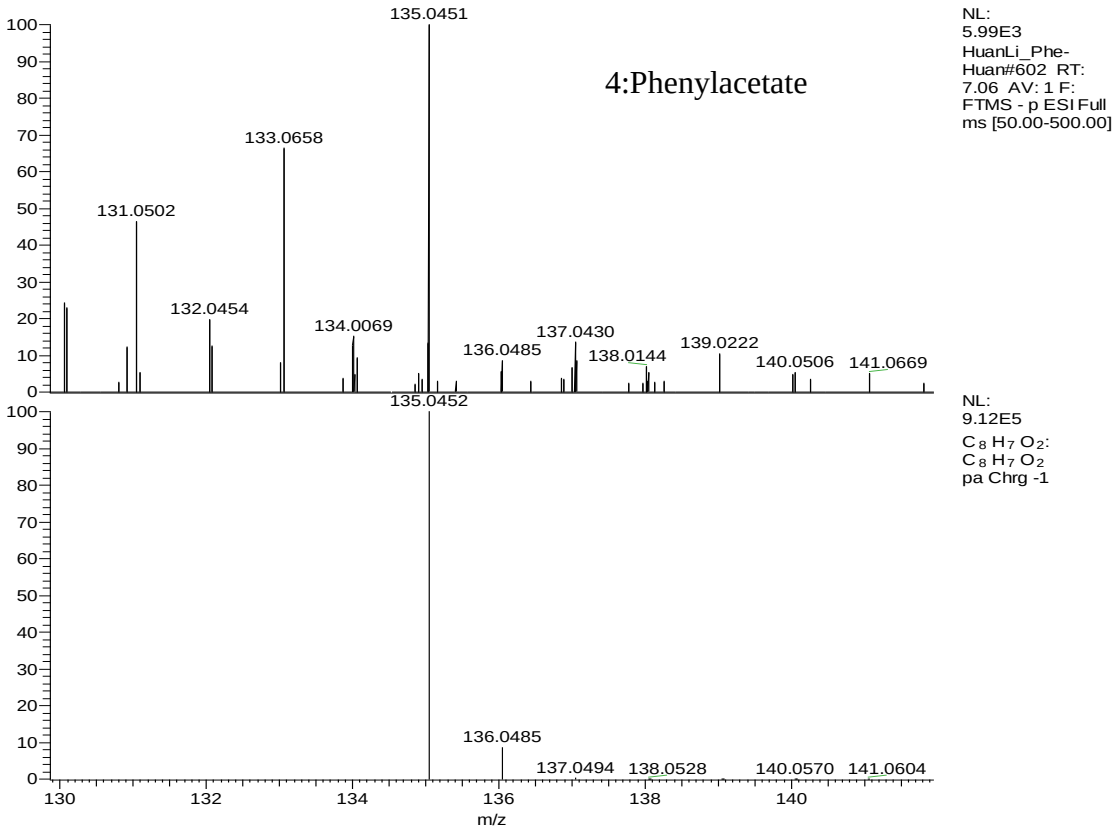
**(b)** Phenylalanine (10 g/L) + Yeast extract (**0.25%**)

| Phenyllactate | Cinnamate | Phenylpropionate | Phenylacetate |
|---|---|---|---|
| + | + | + | + |


1:Phenylalanine
NL:
1.68E5
HuanLi_Phe-Huan#1632 RT: 20.92 AV: 1 F: FTMS - p ESI Full ms [50.00-500.00]
NL:
8.99E5
C9 H10 O2 N1: C9 H10 O2 N1 pa Chrg -1
164.0716
159.0273
162.0018
163.0096
165.0556
167.0614
168.0362
170.0611
164.0717
165.0751
166.0784
167.0793
169.0860
170.0869
m/z


2:Cinnamate
NL:
4.63E4
HuanLi_Phe-Huan#350 RT: 4.33 AV: 1 F: FTMS - p ESI Full ms [50.00-500.00]
NL:
9.03E5
C9 H7 O2: C9 H7 O2 pa Chrg -1
147.0451
140.0716
142.0662
145.0658
146.0458
148.0484
149.0607
151.0638
152.9957
155.0825
157.0982
147.0452
148.0485
149.0519
151.0561
153.0604
m/z


3:Phenylpropionate
149.0607
142.0661
143.0502
146.0610
147.0451
148.0484
150.0641
151.0674
152.9957
156.0818
158.0610
NL:
1.52E5
HuanLi_Phe-Huan#1294 RT: 17.64 AV: 1 F: FTMS - p ESI Full ms [50.00-500.00]
149.0608
150.0642
151.0675
153.0718
155.0760
NL:
9.02E5
C9 H9 O2:
C9 H9 O2
pa Chrg -1
m/z


4:Phenylacetate
135.0451
131.0502
132.0454
133.0658
134.0069
136.0485
137.0430
138.0144
139.0222
140.0506
141.0669
NL:
5.99E3
HuanLi_Phe-Huan#602 RT: 7.06 AV: 1 F: FTMS - p ESI Full ms [50.00-500.00]
135.0452
136.0485
137.0494
138.0528
140.0570
141.0604
NL:
9.12E5
C8 H7 O2:
C8 H7 O2
pa Chrg -1
m/z

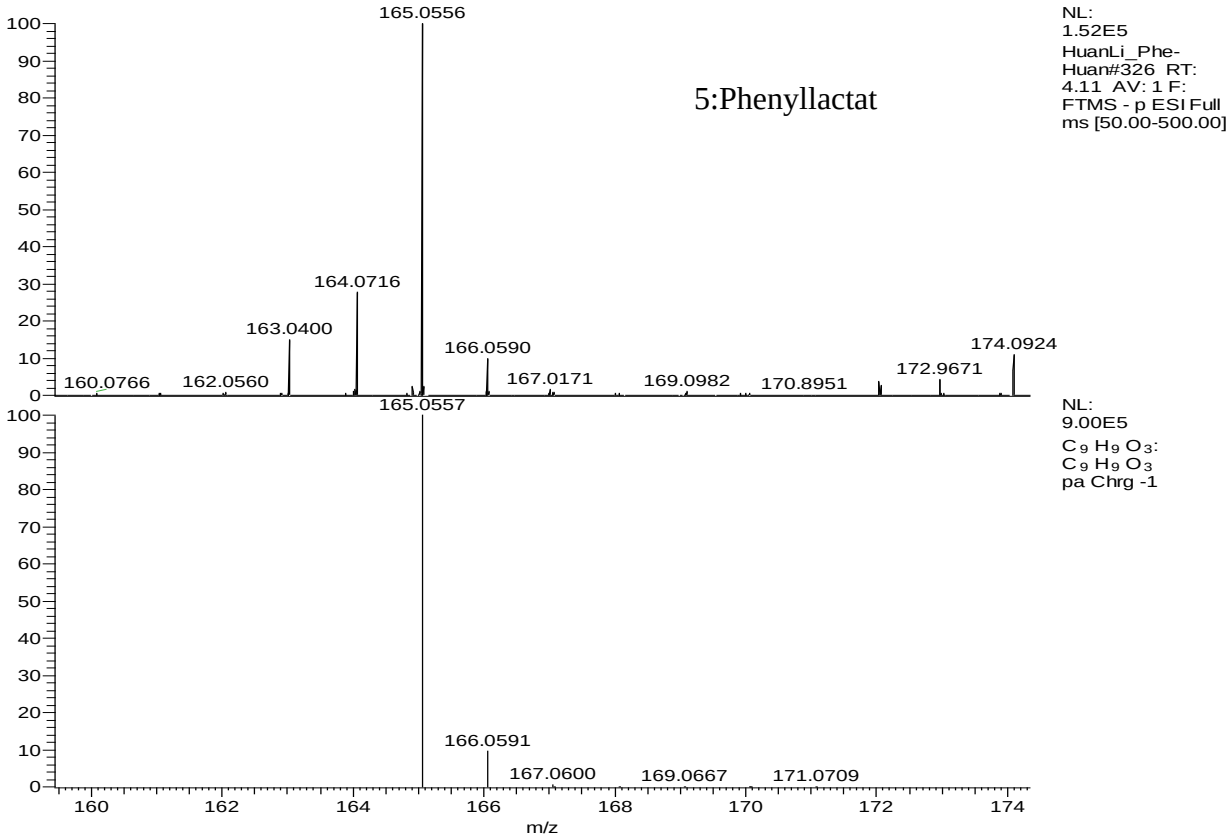
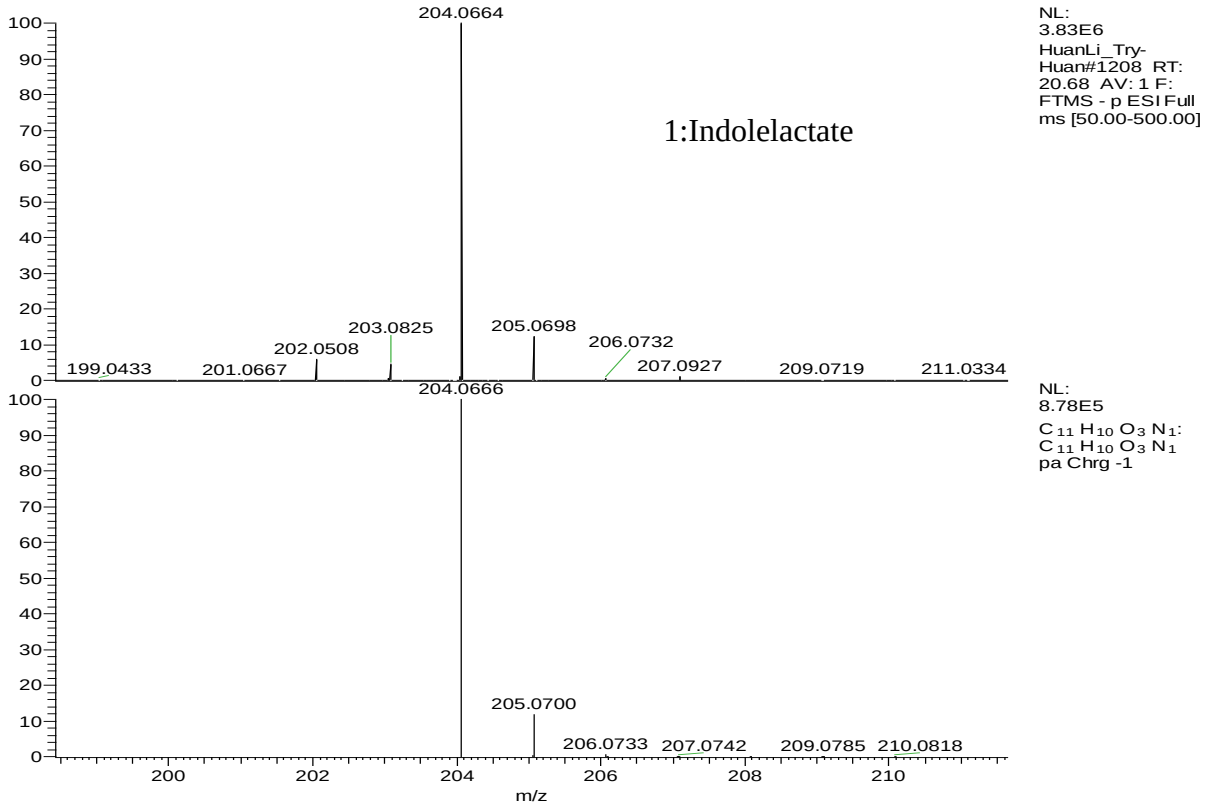
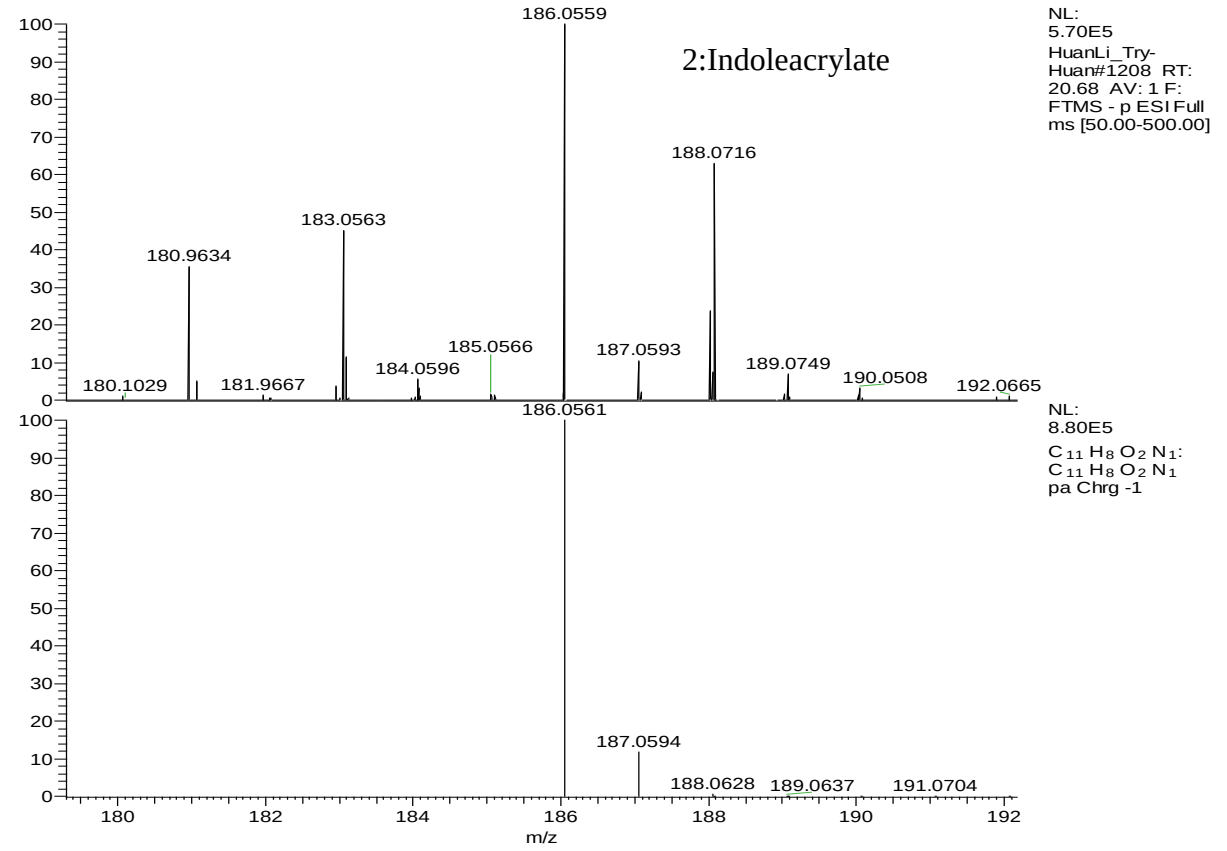
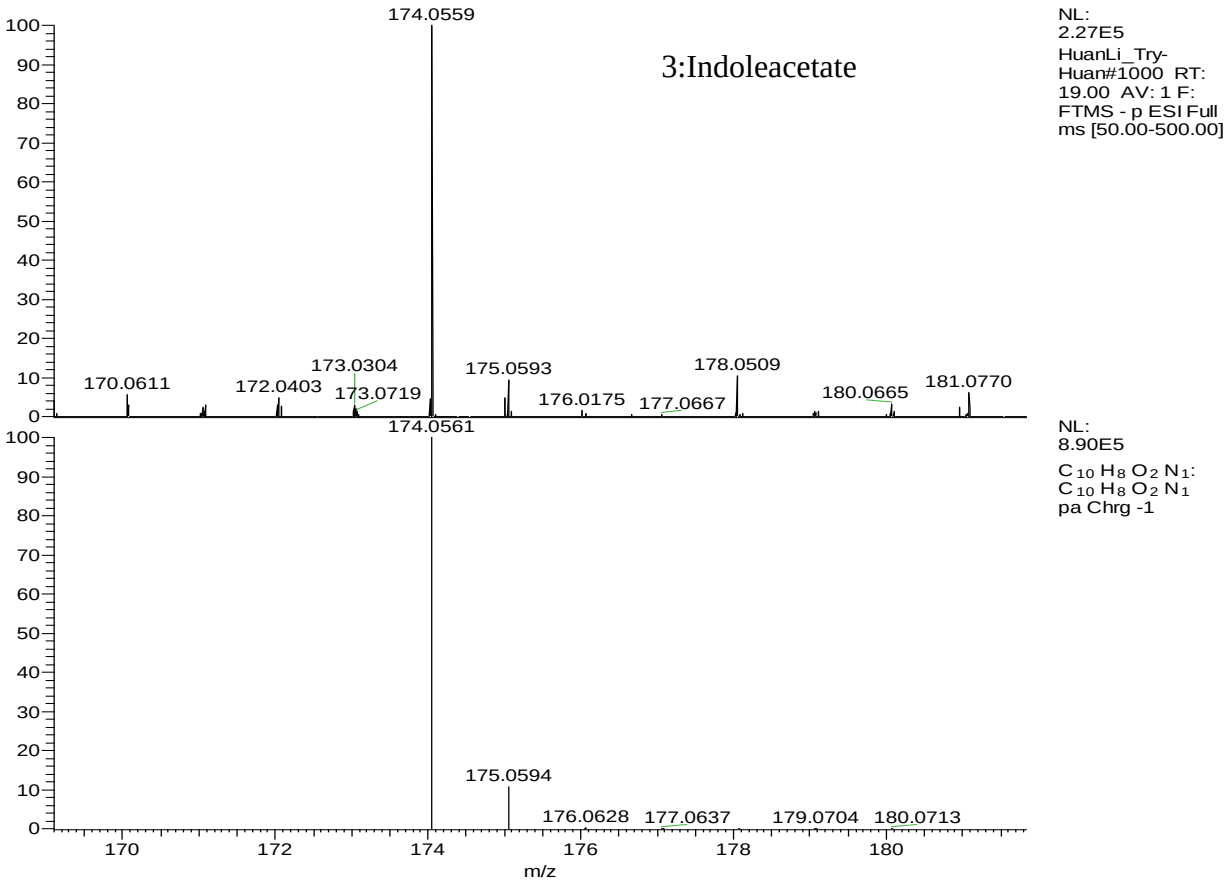



**Fig. 16 (a)** Determination of intermediates and end products from growing cultures on phenylalanine via ESI-MS




2:Indoleacrylate
186.0559
188.0716
183.0563
180.9634
185.0566
187.0593
184.0596
189.0749
190.0508
180.1029
181.9667
192.0665
NL:
5.70E5
HuanLi_Try-Huan#1208 RT: 20.68 AV: 1 F: FTMS - p ESI Full ms [50.00-500.00]
186.0561
187.0594
188.0628
189.0637
191.0704
NL:
8.80E5
C11 H8 O2 N1: C11 H8 O2 N1 pa Chrg -1
m/z


3:Indoleacetate
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173.0304
175.0593
178.0509
170.0611
172.0403
173.0719
176.0175
177.0667
180.0665
181.0770
NL:
2.27E5
HuanLi_Try-Huan#1000 RT: 19.00 AV: 1 F: FTMS - p ESI Full ms [50.00-500.00]
174.0561
175.0594
176.0628
177.0637
179.0704
180.0713
NL:
8.90E5
C10 H8 O2 N1: C10 H8 O2 N1 pa Chrg -1
m/z

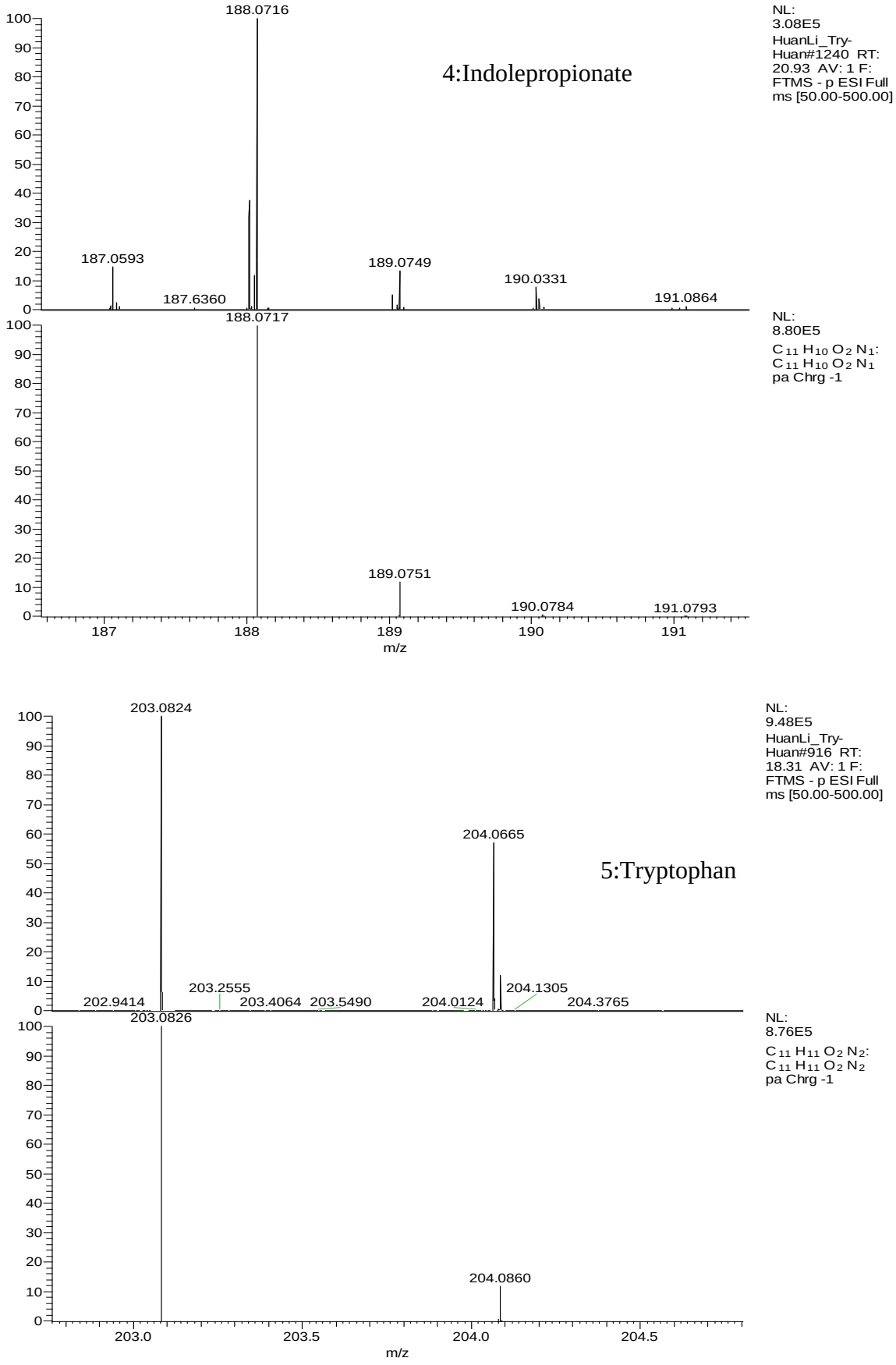
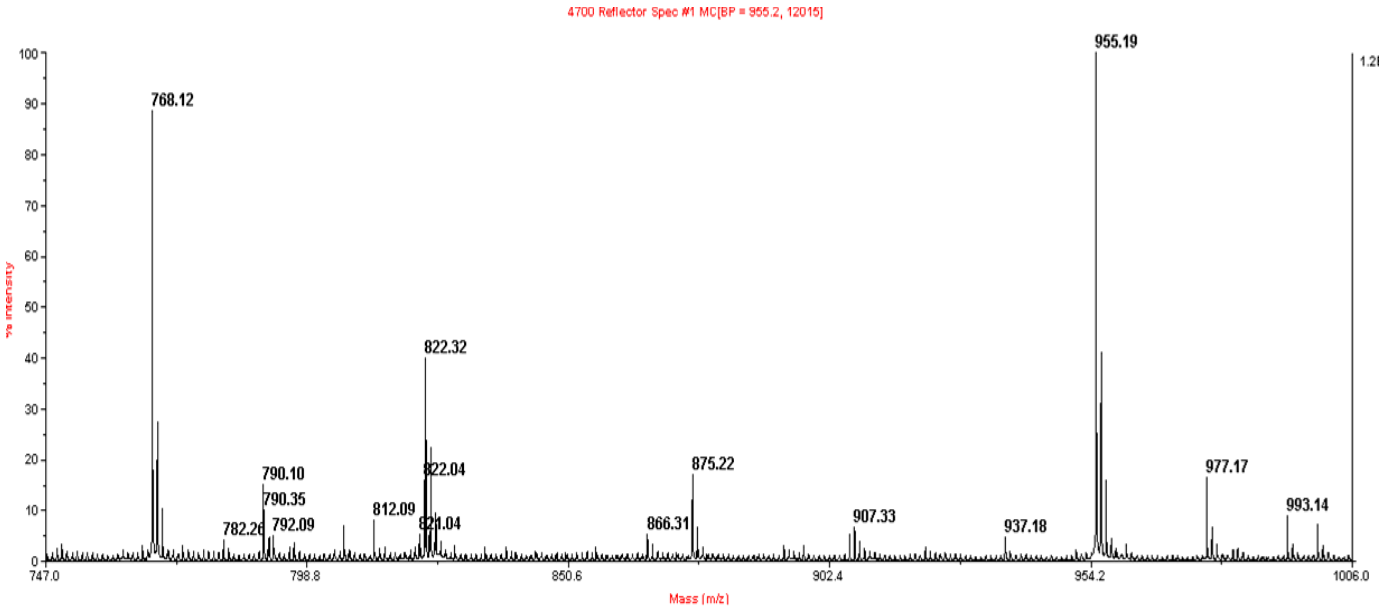



**Fig. 16 (b)** Determination of intermediates and end products from growing cultures on tryptophan via ESI-MS

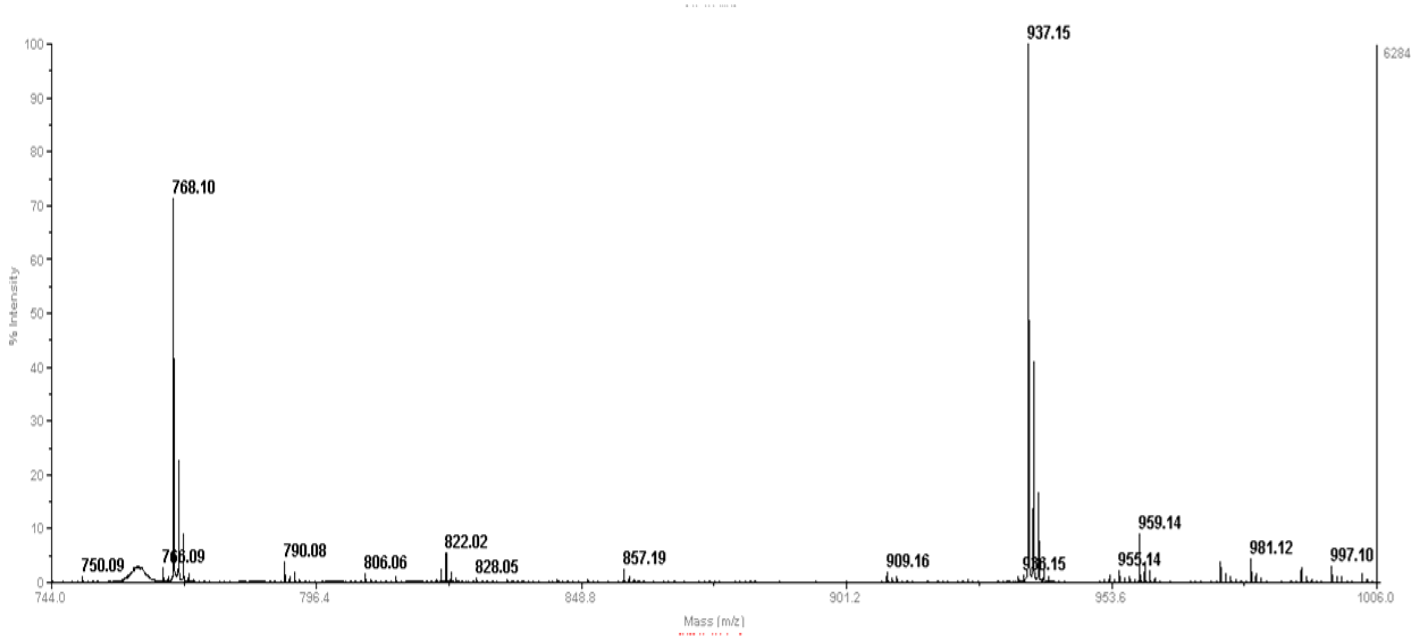
# *2.* Studies on Aryllactyl-CoA dehydratase from C. sporogenes

## *2.1 Chemical synthesis of CoA-thioesters*

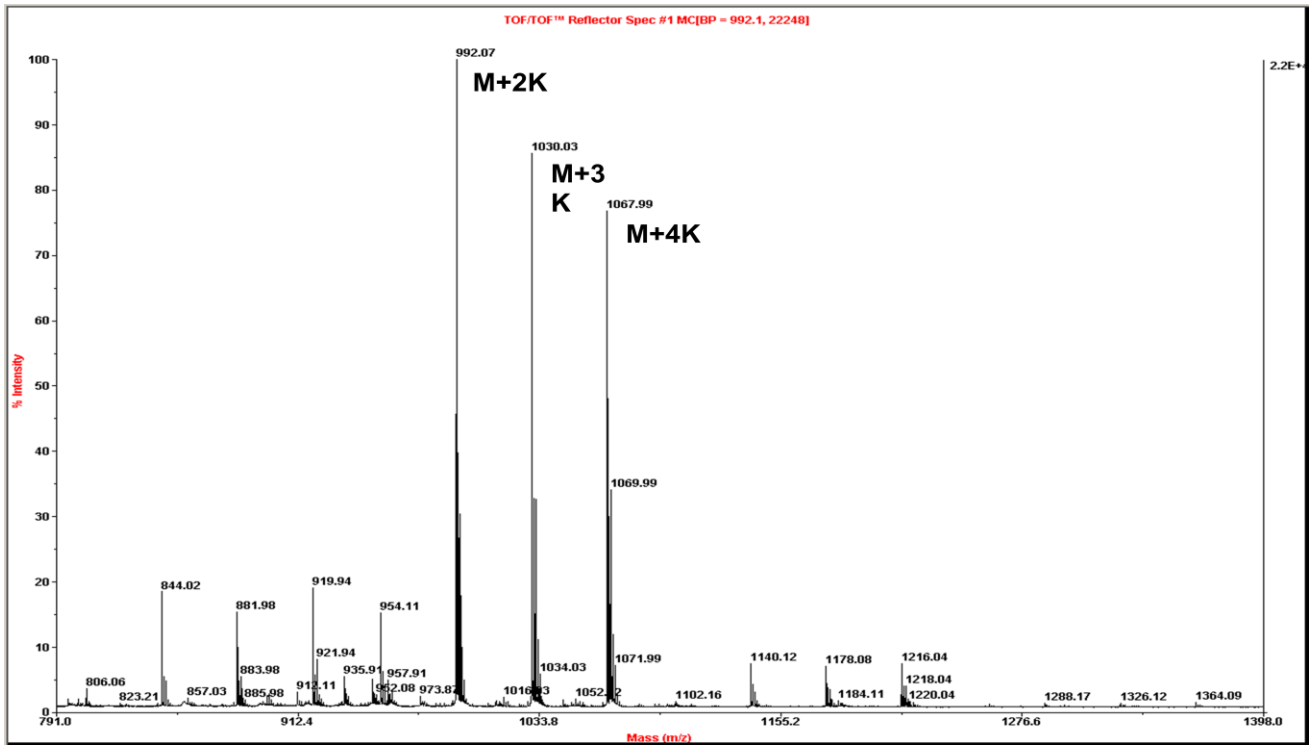
All CoA-thioesters as substrates for the enzyme activity assays were chemically synthesized and confirmed by MALDI-TOF MS (Fig. 17)



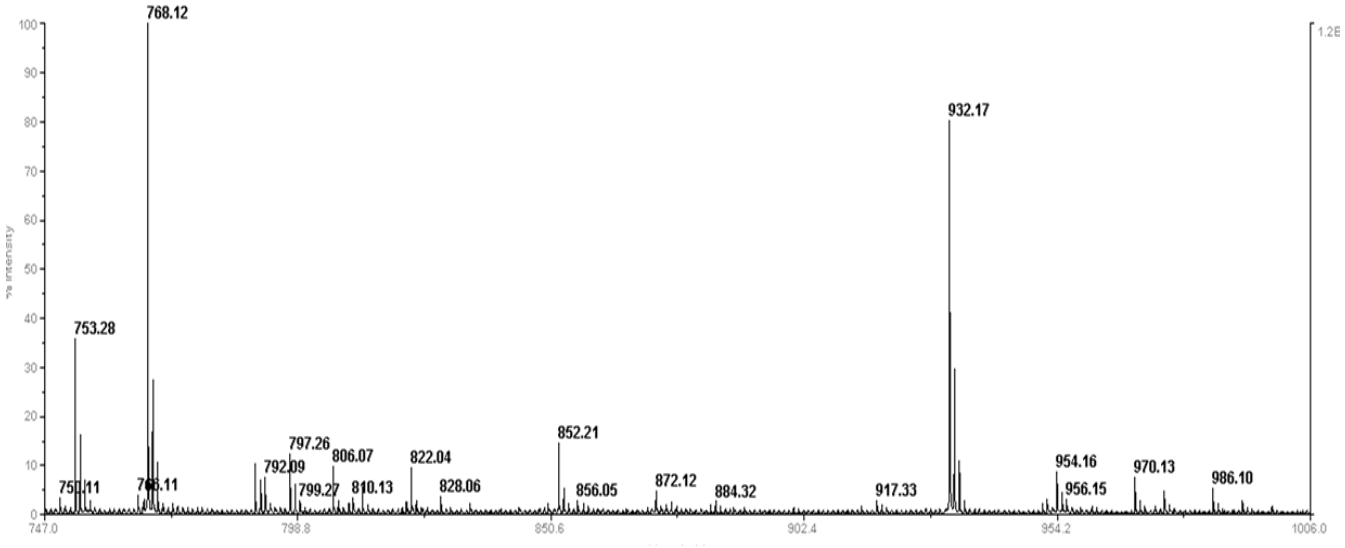

(a)




(b)




(c)




(d)

**Fig. 17** MALDI-TOF mass spectra confirm the molecular masses and purities of the synthesized CoA-thioesters. The peak at 768.10 Da indicated free CoASH (a) (*R*)-3-indollactyl-CoA [M] 956,2 Da, $[M+Na]^+$ 977,2 Da; (b) (*E*)-3-indolacrylyl-CoA [M] 937.3 Da, $[M+Na]^+$ 959.14 Da; (c) (*R*)-phenyllactyl-CoA $[M+2K]^+$ 992.7 Da, $[M+3K]^+$ 1030 Da, $[M+4K]^+$ 1068 Da, the sample was measured in KPP buffer; (d) (*R*)-OH-phenyllactyl-CoA [M] 932.2 Da.

The measurement of the activity of indolelactyl-CoA dehydratase in cell-free extracts at 290 nm ($\varepsilon_{290}$ = 2.2 $mM^{-1}cm^{-1}$) [29], which is the typical absorption of an enoyl-CoA thioester, was not successful. Always a noisy measure line was obtained. The chemical structure shows that indolearcylyl-CoA has an extended π-conjugation system of the CoA thioester, the α-double bond and the large aromatic indolering. This extended π-conjugation system decreases the distance of the energy levels between π and π* orbital resulting in the absorbance at longer wavelengths. The synthesized (*E*)-3-indolacrylyl-CoA shows a very bright yellow color which indicates the expected absorption at longer wavelengths with a peak at 380 nm, $\varepsilon_{380}$ = 6.5 $mM^{-1}cm^{-1}$ (Fig. 18).

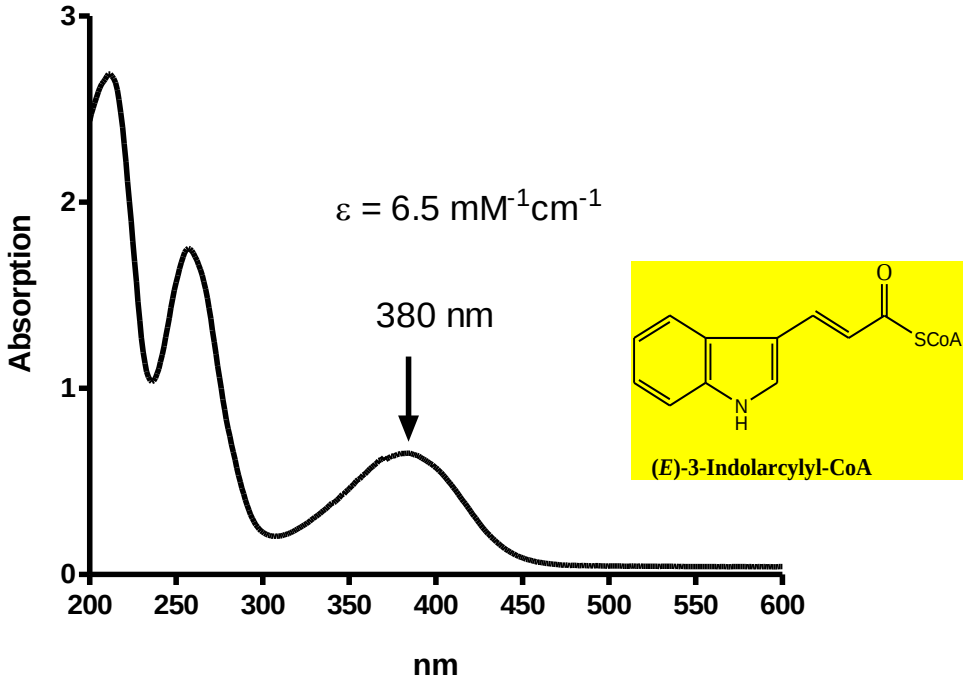



**Fig.18** The UV-visible spectrum of indoleacrylyl-CoA

## *2.2 Purification of the recombinant activator of the dehydratase*

The recombinant activator HgdC of 2-hydroxyglutaryl-CoA dehydratase of *A. fermentans* was purified by Strep-tactin as described in method 3.3.2.4. On SDS-PAGE the purified enzyme showed a single band around 27 kDa (Fig. 19). It has been observed that HgdC could replace the activator FldI for phenyllactyl-CoA dehydratase [8]. Therefore, the purified recombinant HgdC was used for the followed enzyme activity assays of aryllactyl-CoA dehydratases.

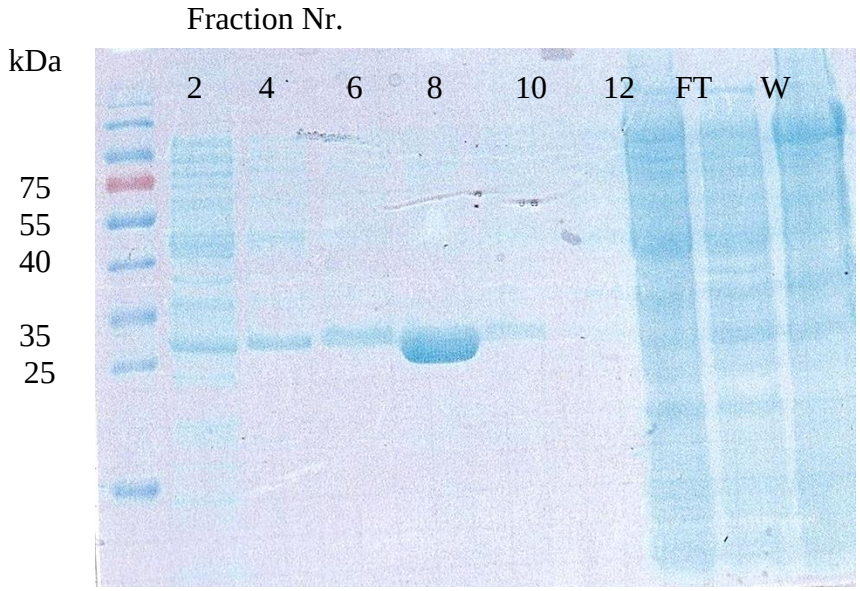



**Fig.19** The SDS-PAGE gel shows the fractions of the purification of HgdC. FT: flow through; W: wash out; CF: cell-free extract.

## *2.3 Purifications of indolelactyl-CoA dehydratase and phenyllactyl-CoA dehydratase*

The indolelactyl-CoA dehydratase from cells grown on tryptophan and the phenyllactyl-CoA dehydratase from cells grown on phenylalanine were purified by three chromatography columns. From the first column, a DEAE Sepharose, the dehydratase was eluted around 0.32 M NaCl by a linear gradient from 0 to 0.6 M NaCl in 20 mM Mops pH 7.3 (Fig. 20). After that the enzymes were eluted from a Phenyl Sepharose column around 0.65 M $(NH_4)_2SO_4$ by a linear gradient from 1 M to 0.3 M $(NH_4)_2SO_4$ in 20 mM Mops pH 7.3 (Fig. 21). At the end the dehydratases were purified to homogenous proteins via a Superdex 200 column and were eluted as a single peak at 75 min (Fig. 22). All fractions which contained the aryllactyl-CoA dehydratases were confirmed by enzyme activity assays and SDS-PAGE. Both dehydratses exhibited enzyme complexes composed of three subunits.

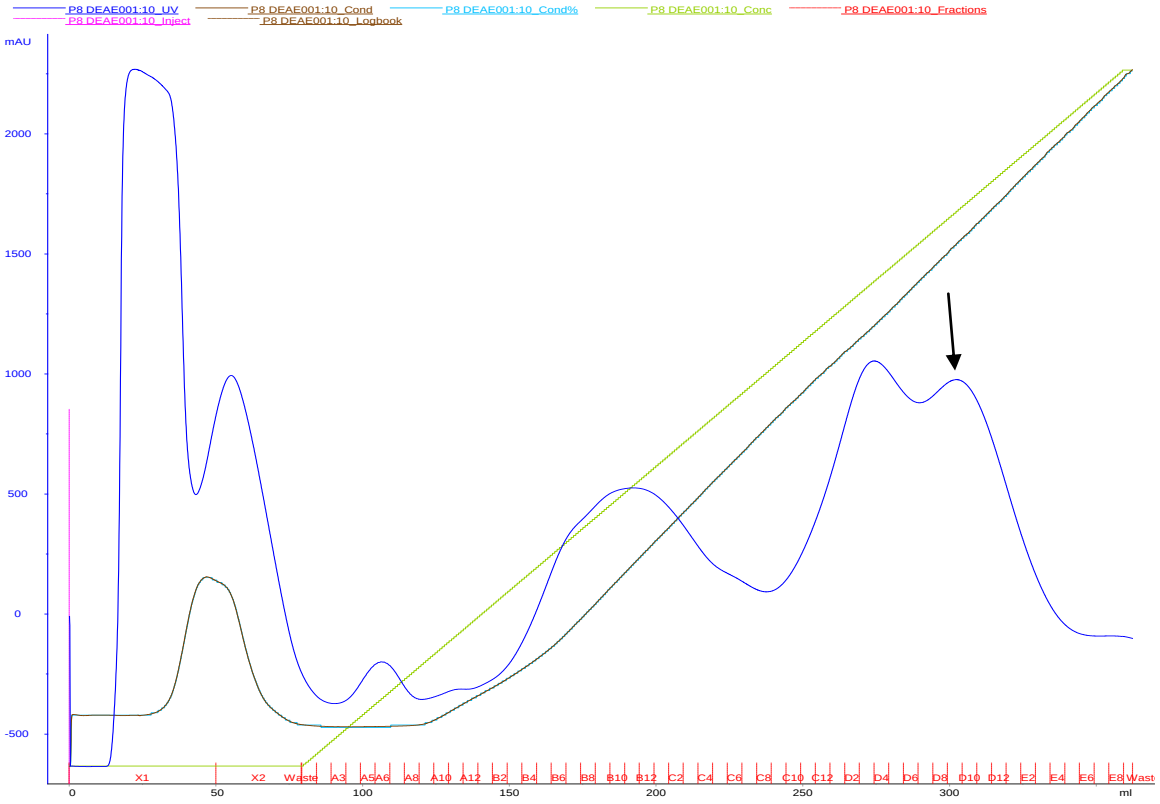



**Fig.20** Elution profile of DEAE cellulose chromatography (blue line: UV absorbance at 280 nm; brown line: conductivity; green line: buffer gradient)

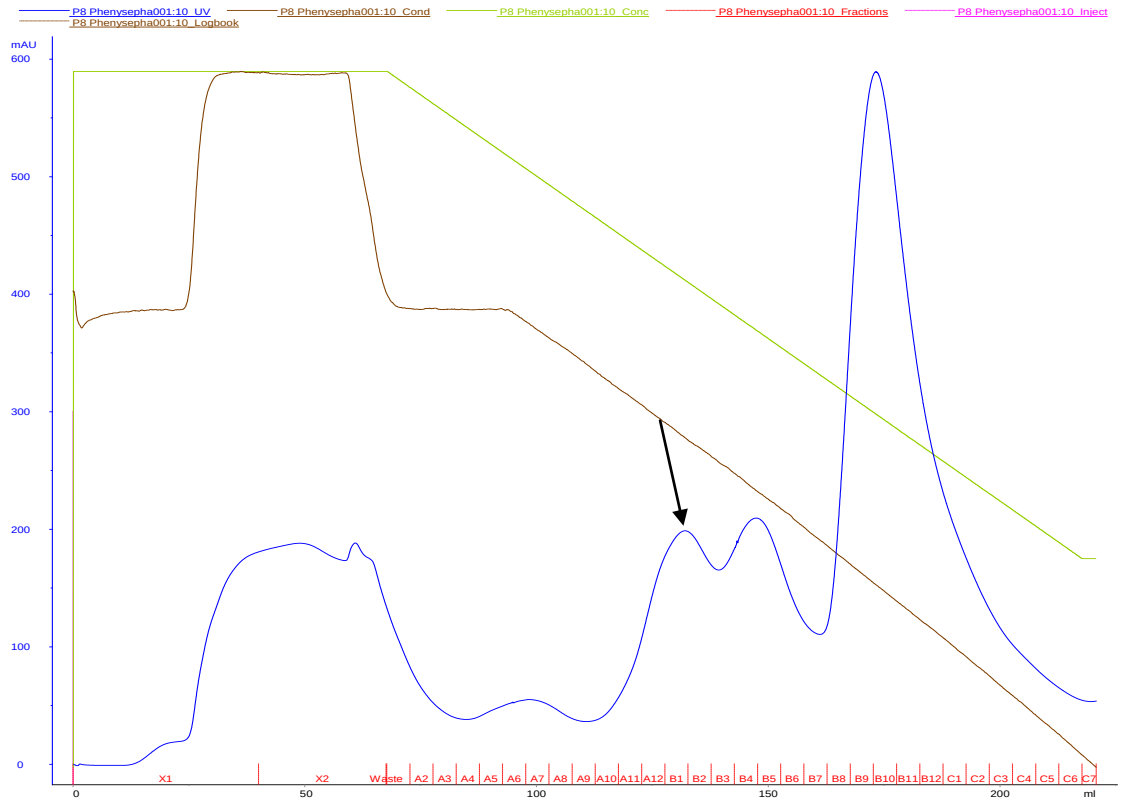



**Fig.21** Elution profile from the phenyl Sepharose column

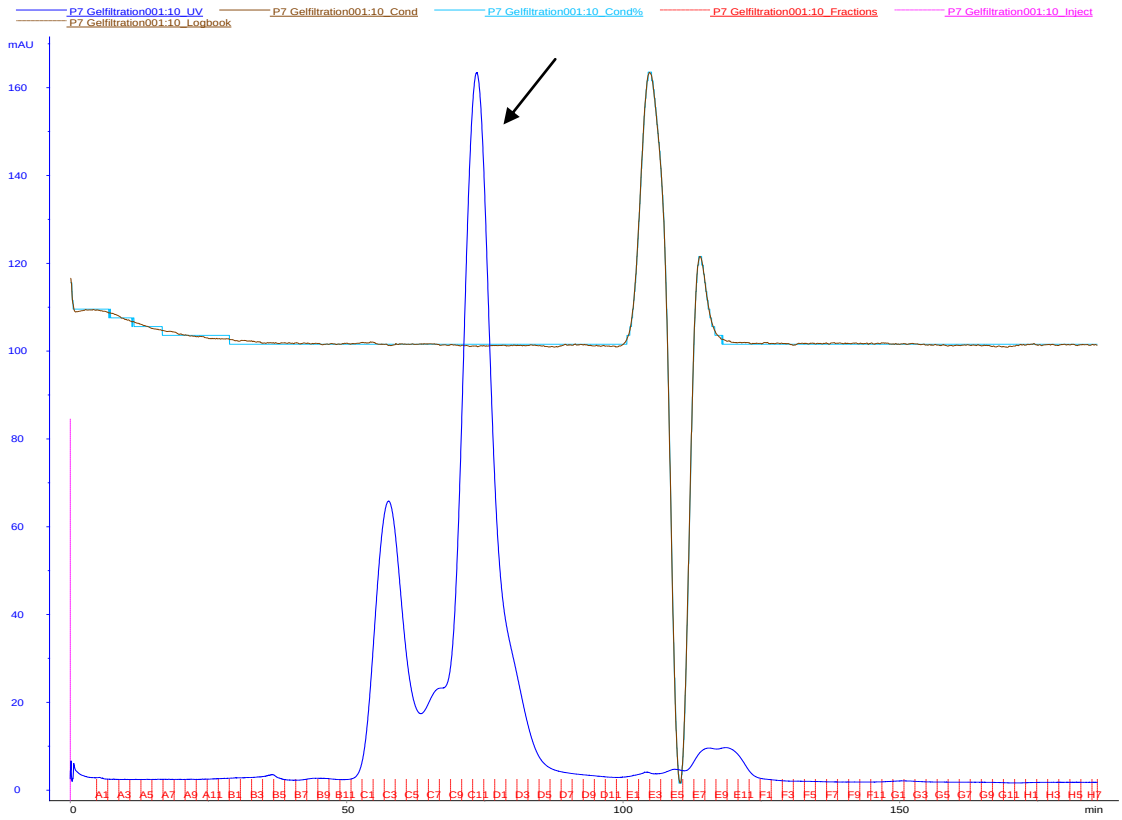



**Fig.22** Elution profile of Superdex200 chromatography

### *2.3.1 The purification of indolelactyl-CoA dehydratase*

The indolelactyl-CoA dehydratase from the first purification shows three bands around 45 kDa, 43 kDa and 40 kDa on SDS-PAGE (Fig. 23a). These three bands were analyzed by MALDI-TOF peptide mass fingerprint and compared with amino acid sequences of the data-bank via Mascot software. The sequences are identical to those of the phenyllactyl-CoA dehydratase complex including the CoA-transferase FldA and the actual dehydratase FldBC, which are encoded by the *fldABC* genes of *Clostridium botulinum* [23]. In chapter 2.8 it will be shown that the *fldBC* genes of *C. sporogenes* and *C. botulinum* Hall strain A [12] are identical. The MALDI-TOF MS spectrum of the first purification confirmed the molecular mass of the A-subunit with 46.4 kDa with that calculated from the gene (46.4 kDa) and that of the C-subunit with 43.2 kDa (calculated 43.1 kDa) but gave a different mass of the B-subunit with 44.4 kDa (calculated 46.2 kDa) (Fig. 23b). The three peaks around 21 kDa, 22 kDa and 23 kDa are also attributed to the dehydratase subunits but twice protonated.

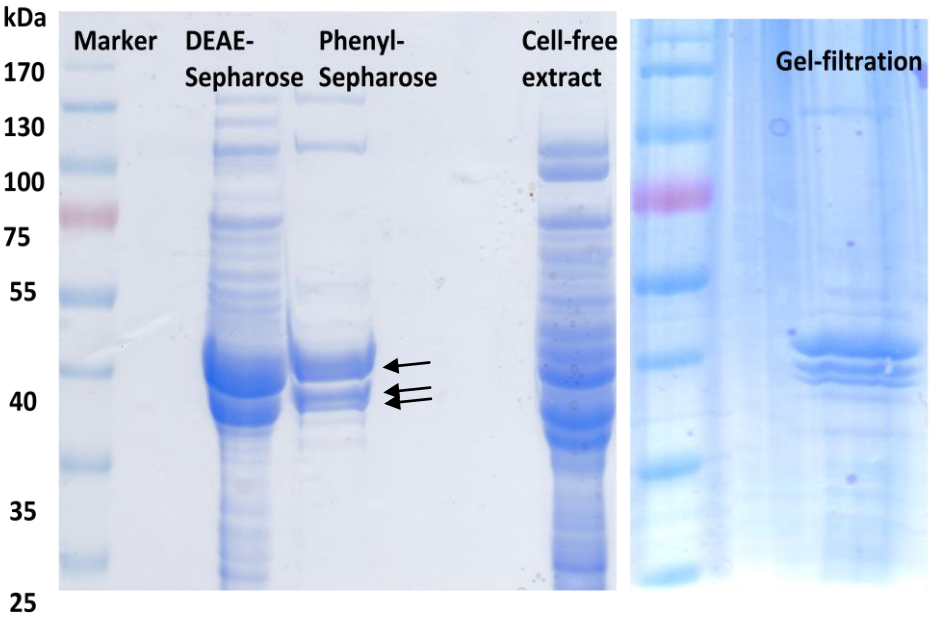
MALDI-TOF MS spectrum of the second purification fraction gave the same molecular mass of the A-subunit with 46.5 kDa, but surprisingly different masses of the B and C-subunits with 43.3 kDa and 38.2 kDa, respectively. The twice protonated B-subunit showed clearly

two peaks with 21.3 kDa and 21.7 kDa (Figs. 23c and d). Later the peptide sequence analysis by Nano LC-MS and Mascot databank identified the 38.2 kDa band as ornithine cyclodesaminase, which did not belong to the dehydratase complex. Probably in the MALDI-TOF MS spectrum only the twice protonated peaks of the B and C-subunits could be separated. The molecular mass of FldC could be calculated from the twice protonated peak as 42.6 kDa.

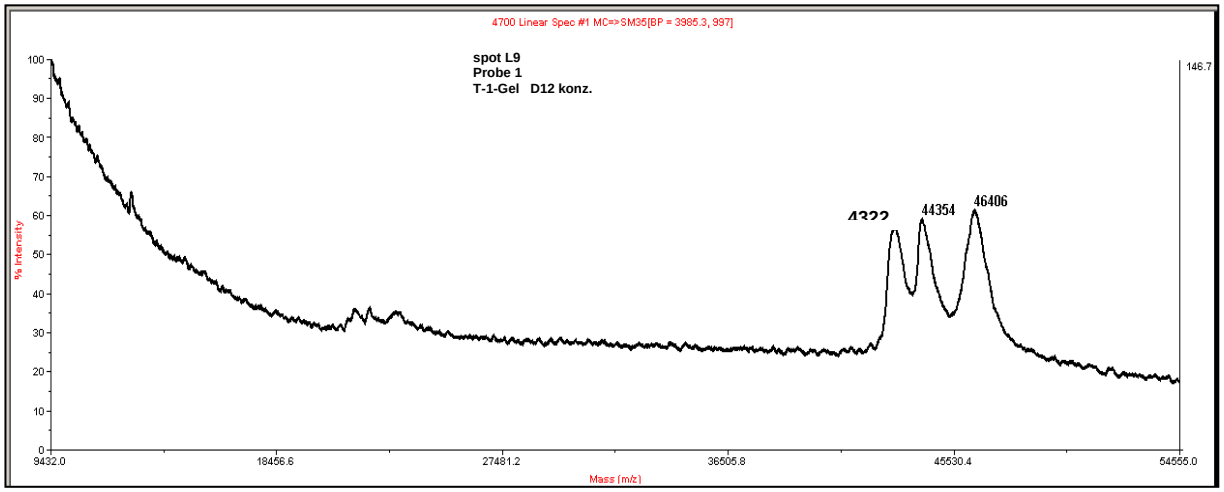
**Table 9** Purification of indolelactyl-CoA dehydratase

| | Spec. Activity * [U/mg] | Yield % |
|---|---|---|
| Cell-free extract | 0.04 | 100 |
| DEAE-Sepharose | 0.15 | 75 |
| Phenyl-Sepharose | 0.8 | 50 |
| Gel-filtration | 3.6 | 30 |

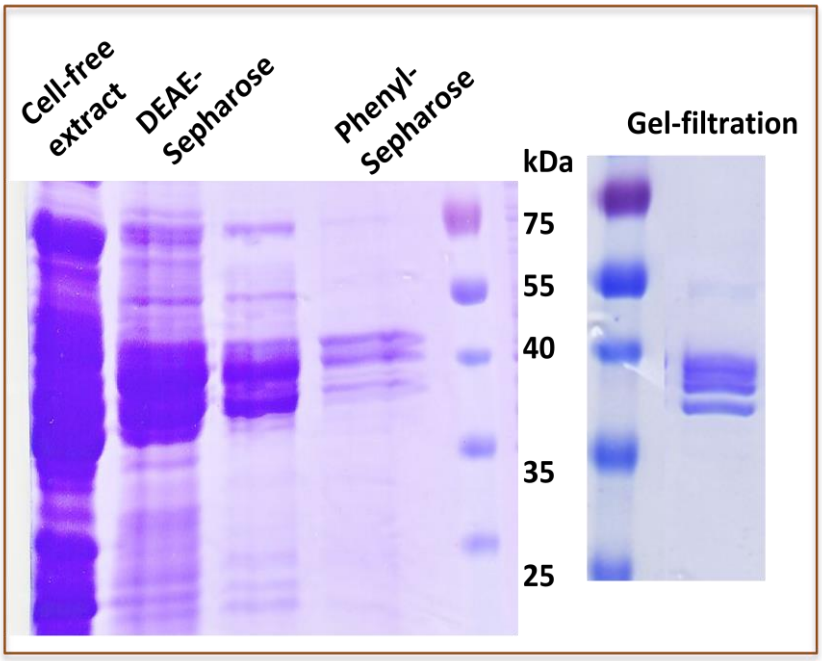
* Determination with 5 µM indolelactyl-CoA




(a) SDS-PAGE of the first purification of indolelactyl-CoA dehydratase




(b) MALDI-TOF MS of indolelactyl-CoA dehydratase from the first purification




(c) The second purification of indolelactyl-CoA dehydratase

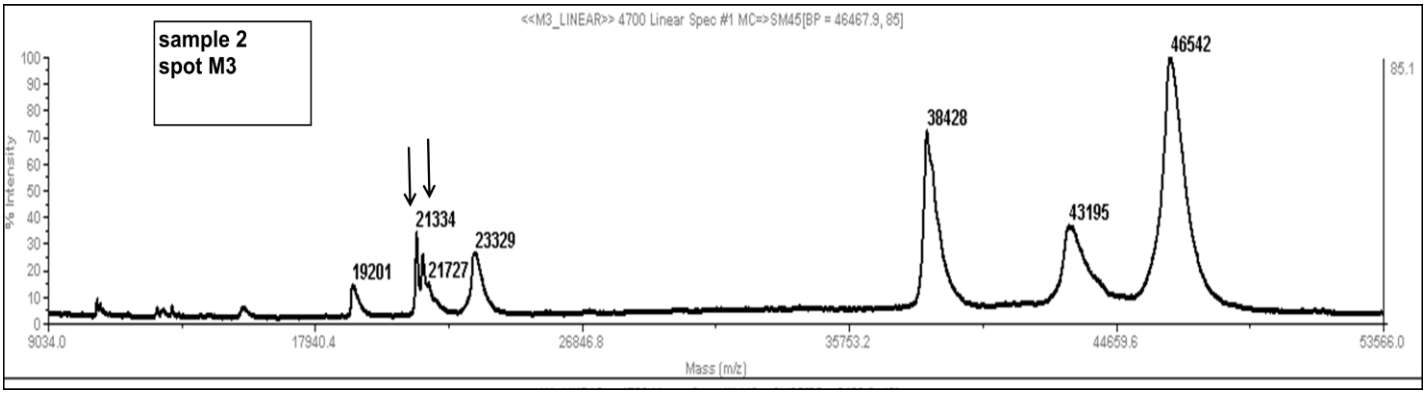



**Fig.23** (d) MALDI-TOF MS of indolelactyl-CoA dehydratase from the second purification

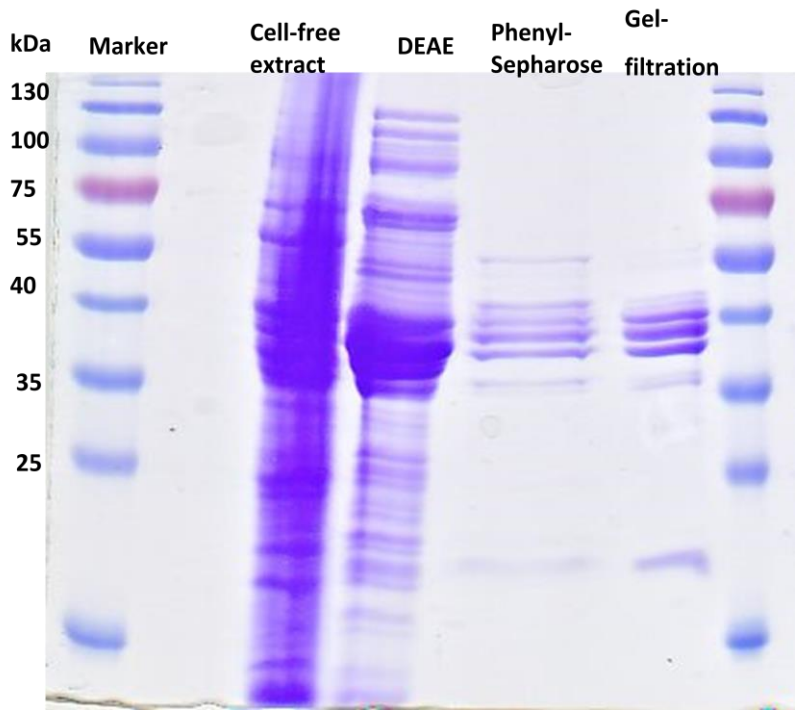
### *2.3.2 The purification of phenyllactyl-CoA dehydratase*

The SDS-PAGE bands of the purified phenyllactyl-CoA dehydratase (Fig. 24a und c) were also analyzed by MALDI-TOF MS. The peptide sequences revealed that phenyllactyl-CoA dehydratase and indolelactyl-CoA dehydratase were identical enzymes. The molecular mass of the large subunit was around 46.5 kDa. The medium and the small subunits from the first purification showed also an overlapping peak at 43.2 kDa and two peaks around 22 kDa in the area of twice protonated masses similar to the phenomenon observed with indolelactyl-CoA dehydratase. The band at around 33 kDa in Fig. 24c was identified as fructose-1, 6-bisphosphate aldolase. The subunits from another purification together with protease inhibitor cocktail (Sigma-Aldrich, Germany) showed again an overlapping peak around 43 kDa with two shoulders (Fig. 24d). The ornithine desaminase was also present. The peak at 41.9 kDa was later identified by Nano LC-MS as oxidoreductase of the NAD-binding Rossmann fold family.

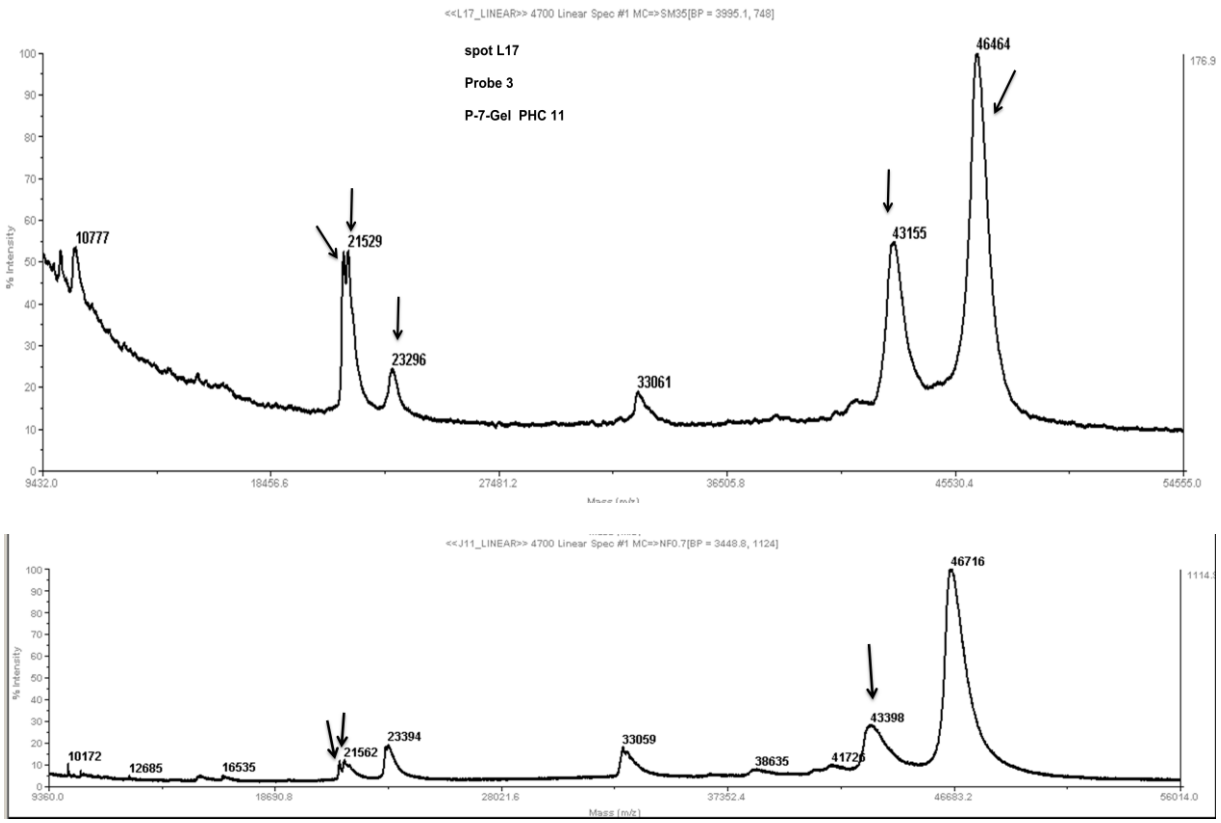
**Table 10** Purification of phenyllactyl-CoA dehydratase

| | Spec. Activity * [U/mg] | Yield % |
|---|---|---|
| Cell-free extract | 0.01 | 100 |
| DEAE-Sepharose | 0.1 | 80 |
| Phenyl-Sepharose | 0.36 | 62 |
| Gel-filtration | 0.74 | 34 |

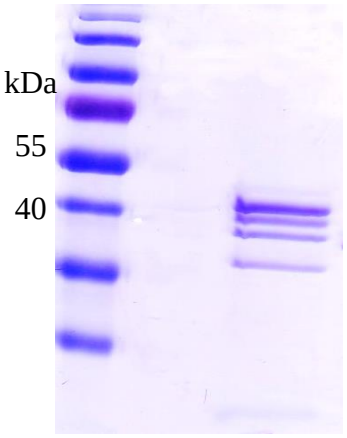
* Determination with 10 µM phenyllactyl-CoA




(a) The first purification of phenyllactyl-CoA dehydratase




(b) The tow MALDI-TOP MS of phenyllactyl-CoA dehydartase from the first purification




(c) The second purification of phenyllactyl-CoA dehydartase

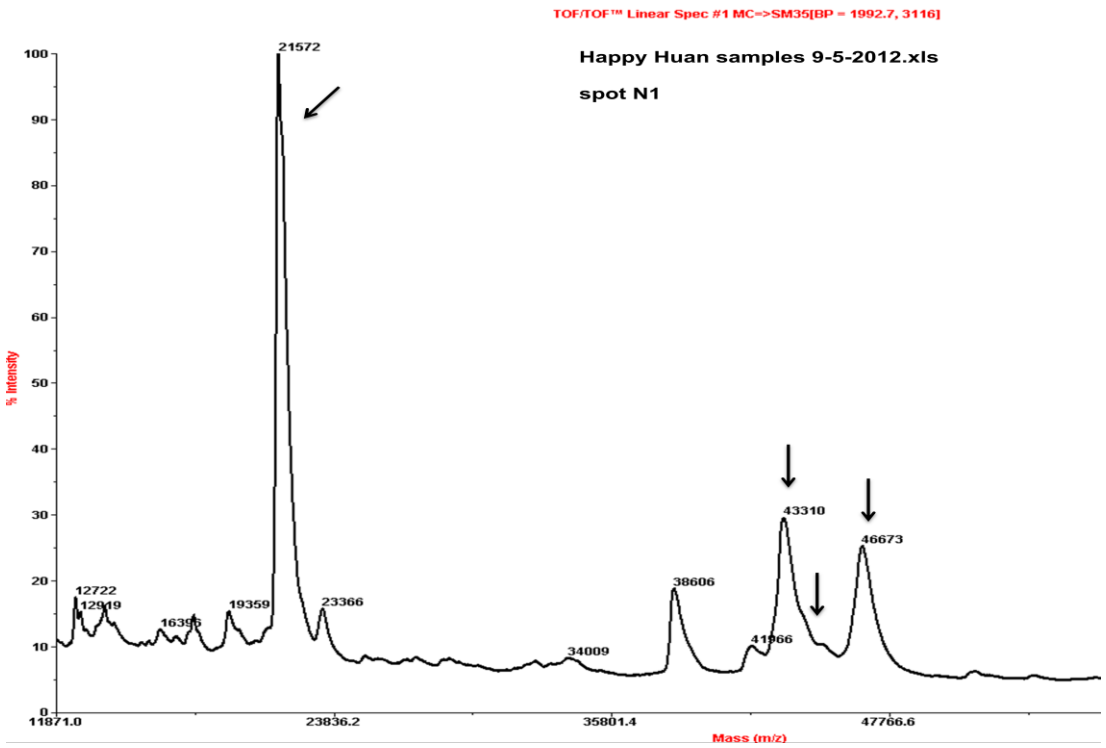



**Fig. 24** (d) MALDI-TOF MS of phenyllactyl-CoA dehydratase from the second purification

## *2.4 Enzyme activities of aryllactyl-CoA dehydratase*

The enzyme activities were measured directly with the chemically synthesized aryllactyl-CoA substrates. In previous experiments it was determined that the dehydration reaction only occurred at the CoA-thioester level. The measurement of activity was followed at 380 nm with indolelactyl-CoA and at 310 nm with phenyllactyl-CoA and *p*-OH-phenyllactyl-CoA. After thawing in the anaerobic chamber, the cell-free extracts required at least 20 - 30 min at room temperature until full activity was observed.

The highest specific activities $V_{max}$ of the aryllactyl-CoA dehydratases in cell-free extracts were obtained with that aryllactyl-CoA derived from the amino acid, on which the cells were grown (Table 11). The same was true for the purified enzymes (Table 12).

**Table 11** Comparison of dehydratase activities in cell-free extracts (CFE)

| $V_{max}$ (mU/mg) Substrate | CFE from Phe-culture | CFE from Trp-culture |
|---|---|---|
| Indolelactyl-CoA | 6 | 39 |
| Phenyllactyl-CoA | 29 | 23 |

**Table 12** Activities of the purified dehydratases

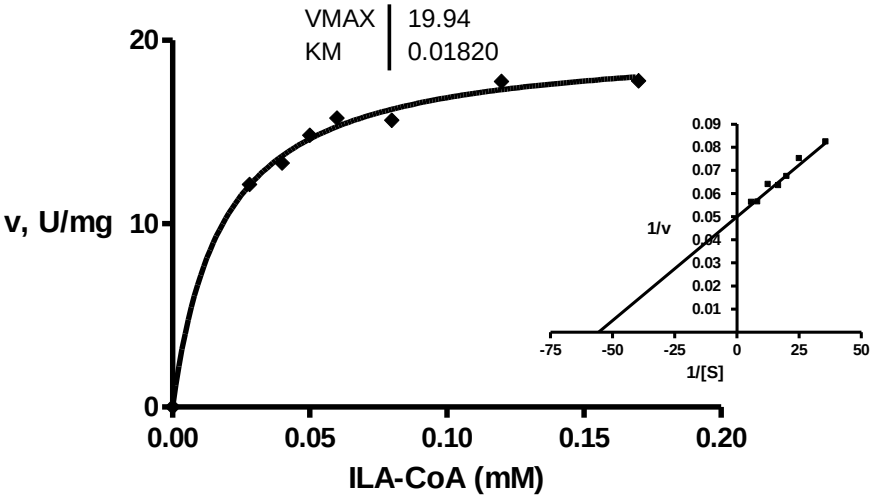
| $V_{max}$ (U/mg) Substrate | Dehydratase from Phe-culture | Dehydratase from Trp-culture |
|---|---|---|
| Indolelactyl-CoA | 7 ± 0.4 | 20 ± 0.5 |
| Phenyllactyl-CoA | 15 ± 1 | 11 ± 1 |
| p-OH-Phenyllactyl-CoA | 0.3 | 0.1 |

The activities measurements of both purified aryllactate dehydratases in the forward and reverse directions gave smooth Michaelis-Menten curves and Lineweaver-Burk-Diagramms (Fig. 24 and 25), from which $K_m$ and $V_{max}$ could be calculated. Table 13 and Table 14 summarize the kinetic data and show that the dehydration of aryllactyl-CoA to arylenoyl-CoA is reversible and the reaction prefers to produce arylenol-CoA which contains conjugated π-system of the double bond and the carbonyl group with the aromatic ring. The equilibrium constant $K'$ of the reaction from indolelactyl-CoA to indolearcylyl-CoA was calculated using the Briggs-Haldane equation as 74 and that of the reaction from phenyllactyl-CoA to cinnamoyl-CoA as 51.

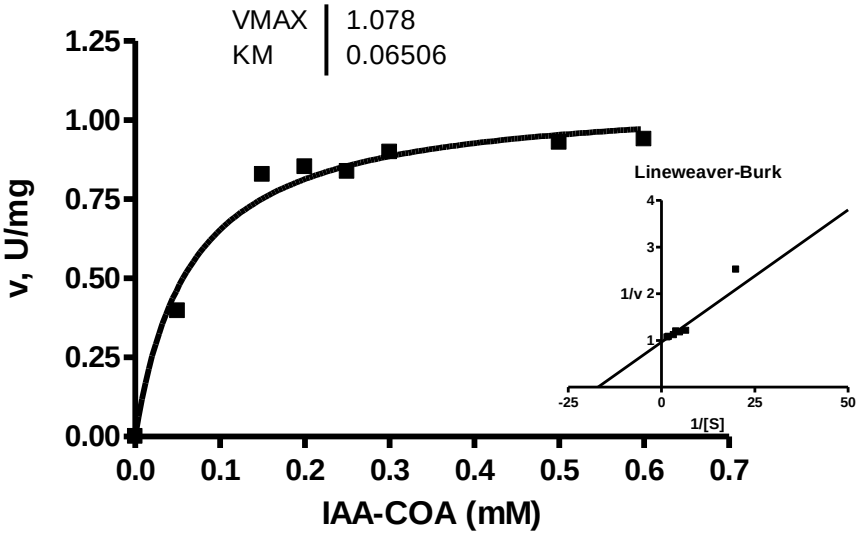
**Table 13** Kinetic measurements of the dehydratase from Trp-culture

| Dehydratase from Trp-culture | $K_m$ (mM) | $V_{max}$ (U/mg) | $V_{max}/K_m$ (U $mg^{-1}$ $mM^{-1}$) |
|---|---|---|---|
| Indolelactyl-CoA | 0.018 ± 0.002 | 19.9 ± 0.5 | 1106 |
| Indoleacrylyl-CoA | 0.07 ± 0.01 | 1.08 ±0.03 | 15 |

***Equilibrium constant: K' = ($V_{max1}$ /$K_{m1}$) / ($V_{max2}$ /$K_{m2}$) = 74***




(a) Indolelactyl-CoA as substrate



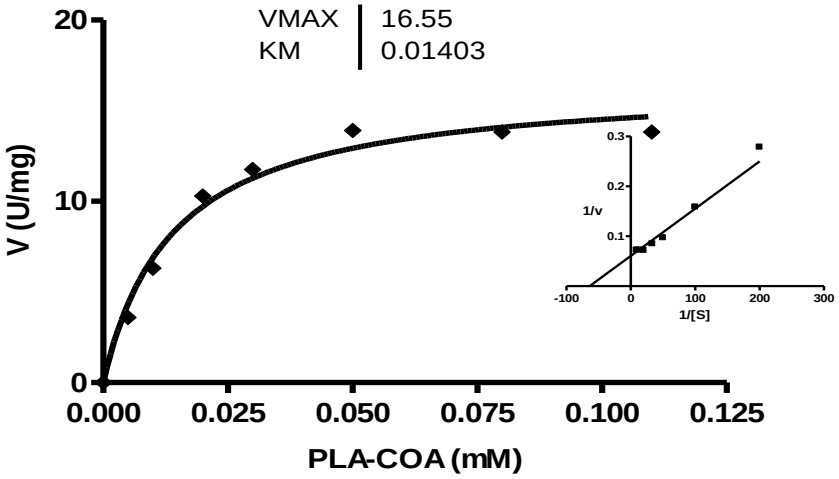

(b) Indolearcylyl-CoA as substrate

**Fig. 24** Kinetic data of dehydratase purified from Trp-culture in Michaelis-Menten graph and Lineweaver-Burk plot

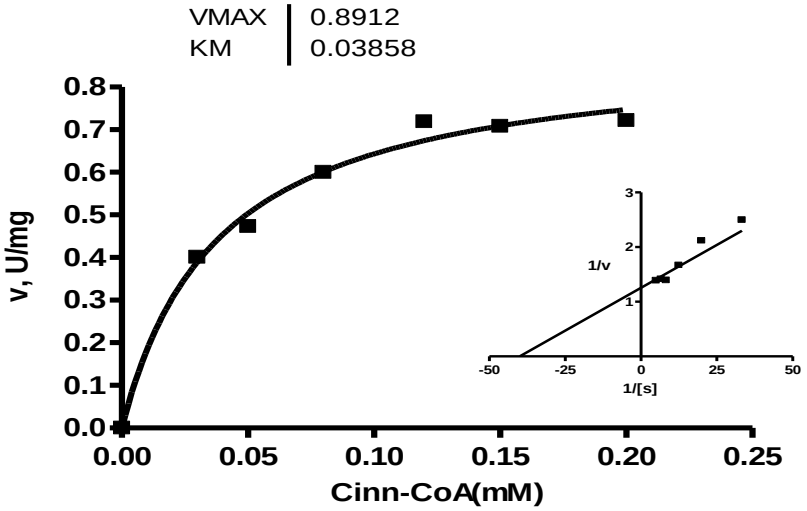
**Table 14** Kinetic measurements of the dehydratase from Phe-culture

| Dehydratase from Phe-culture | $K_m$ (mM) | $V_{max}$ (U/mg) | $V_{max}/K_m$ (U $mg^{-1}$ $mM^{-1}$) |
|---|---|---|---|
| Phenyllactyl-CoA | 0.014 ± 0.002 | 16.6 ± 0.8 | 1186 |
| Cinnamoyl-CoA | 0.039 ± 0.006 | 0.89 ± 0.05 | 23 |

***Equilibrium constant:*** $K' = (V_{max1}/K_{m1}) / (V_{max2}/K_{m2}) = 51$




(a) Phenyllactyl-CoA as substrate




(b) Cinnamoyl-CoA as substrate

**Fig.25** Kinetic data of the dehydratase purified from Phe-culture in Michaelis-Menten graph and Lineweaver-Burk plot

## *2.5 Purification of phenyllactyl-CoA with protease inhibitor and native gel*

Both FldB of the dehydratase complexes from the purified enzymes are smaller than calculated from the molecular masses derived from the genes. To determinate a possible effect of proteases on the size difference of the dehydratases, the enzyme was purified again from a Phe-culture with addition of a protease inhibitor cocktail. But the mass of both subunits remained the same as before. The enzymes after purification with protease inhibitor (P-8) and without (P-7) protease inhibitor were also analyzed on a native gel (Fig. 26). Two bands with masses of 100 and 40 kDa were observed, whereas the expected mass with all three subunits was 130 kDa. The bands were sent to sequence analysis via MALDI-TOF. The band at 100 kDa contained all the three subunits of the enzyme complex. Surprisingly, the band around 40 kDa was not the CoA-transferase FldA but the small subunit FldC, which was separated from the enzyme complex during the running through the native gel.

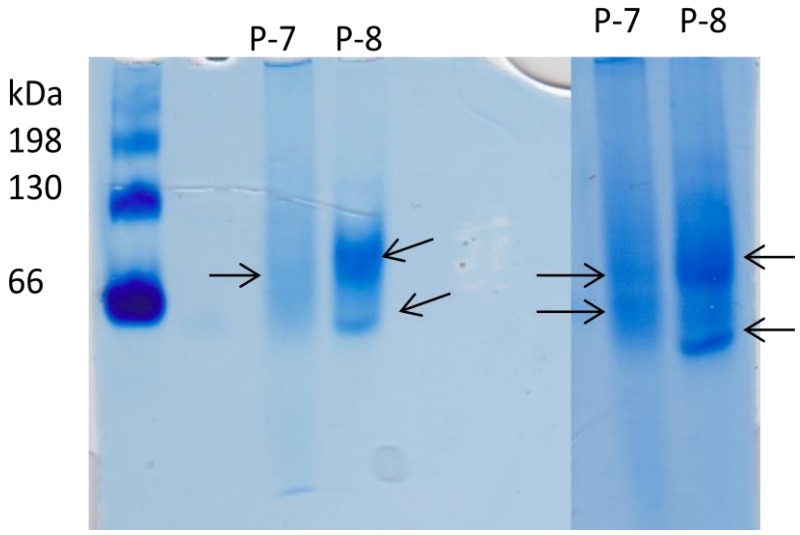



**Fig.26** Native gel of the dehydratase purified from Phe-culture with (P-8) and without (P-7) protease inhibitor cocktail

## *2.6 Time and temperature influence*

In previous experiments was observed that the thawed purified dehydratases became active only after staying at room temperature for at least 20 minutes. At 37 °C maximum activity of aryllactyl-CoA dehydratase was reached within 10 min. The same activity was obtained at 60 °C. At 65 °C the dehydratase rapidly lost its activity. The optimal reaction temperature for aryllactyl-CoA dehydratase is 37 °C (Fig. 27).

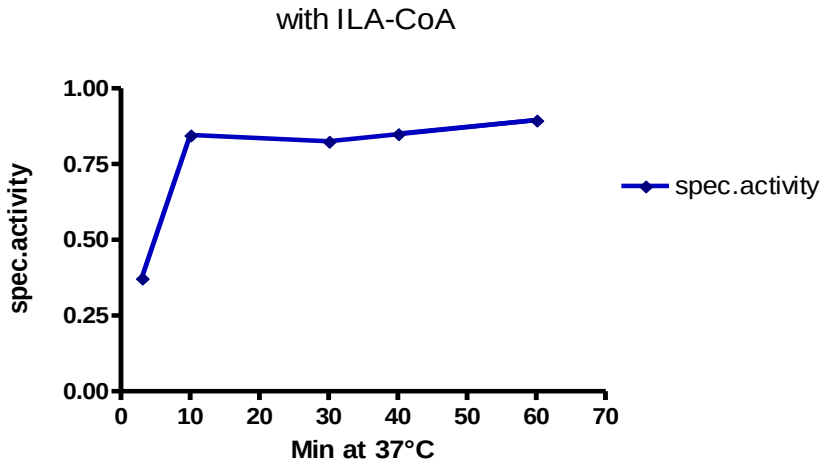



**Fig. 27** The optimal temperature for activity of the dehydratase is at 37 °C.

## *2.7 Complex fixing with $AlF_4$*

In the process of electron transfer a conformational change occurs in activator by ATP, which probably opens the helix-cluster-helix angle from 105° to 180° [19, 28, 29]. $AlF_4^-$ together with ADP inhibited the activation by forming an isolable complex between dehydratase and its activator. Based on the investigation of stabilization of the two-component complex system of 2-hydroxyisocaproyl-CoA dehydratase and its activator from *Clostridium difficile* by $AlF_4^-$ × ADP [19, 29], the possibility of complex building of aryllactyl-CoA dehydratase from *C. sporogenes* with activator HgdC was also tested via size-exclusion chromatography Superdex 200 column. After anaerobic incubation of dehydratase and activator with fresh $AlF_4^-$ × ADP at room temperature for 60 minutes there is a peak at 71.97 minute eluted before dehydratase at 74.96 minute (Fig. 28), the same was also observed with recombinant FldBC and activator.

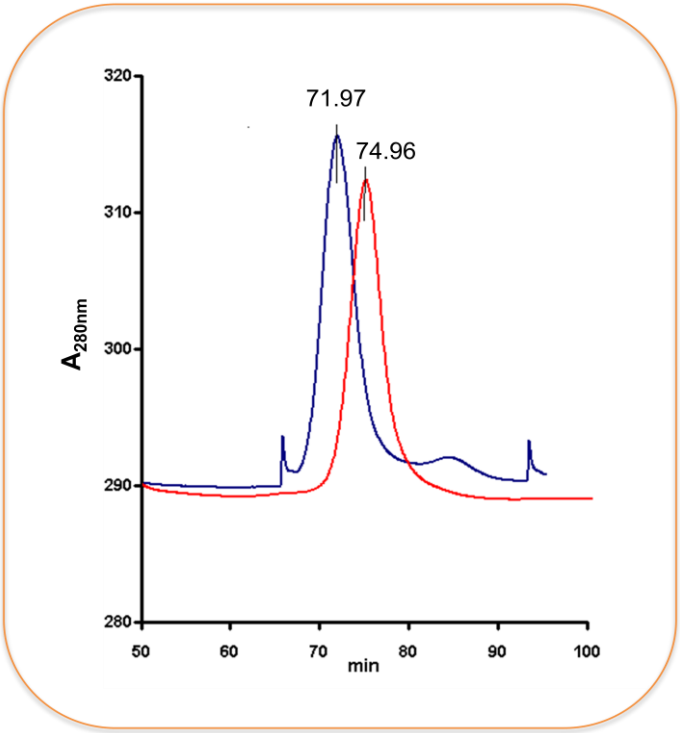



**Fig.28** Recombinant aryllactyl-CoA dehydratase FldBC (red, elution at 75 min). FldBC-activator complex with $AlF_4^-$ (blue, elution at 72 min)

## *2.8 Cloning and expression of fldBC in E. coli*

The genes *fldBC* encoding phenyllactyl-CoA dehydratase was amplified by PCR using Phusion DNA polymerase and the isolated genomic DNA from *C. sporogenes* grown on tryptophan as template. After digestion the PCR product was introduced into a pASK-IBA7plus vector (Fig. 29). The *E. coli* BL21cells containing *fldBC* were first grown anaerobically at 37 °C in 3 serum bottles each with 100 ml Standard I-medium containing 2 mM Fe(III)citrate, 1.3 mM $FeSO_4$, 1 mM $(NH_4)$Fe(III)citrate and 1 mM cysteine as iron-sulfur cluster sources. When the growth reached $OD_{598}$ = 0.5 – 0.6 the cultures were induced with AHT (100 µg/l) and incubated further either for 2 hours, 4 hours or overnight. The cells were harvested and opened as described in Methods. The cell-free extracts and pellets from each sample were loaded on SDS-PAGE to check the expression of the dehydratase genes (Fig. 30). Most of the obtained FldBC was present in the soluble cell-free extract. The yield of the samples from the overnight cultures was highest. But when the *E. coli* BL21cells containing *fldBC* were grown anaerobically in a big volume of 2 l or 3 l, the induction at 37 °C for overnight yielded a very low production of the enzyme. Therefore, the *E. coli* cells were grown aerobically in 3 l Standard I-medium containing extra iron and sulfur sources at 37 °C

to $OD_{598}$ around 0.6 and induced with AHT. After induction the culture was incubated further at room temperature overnight.

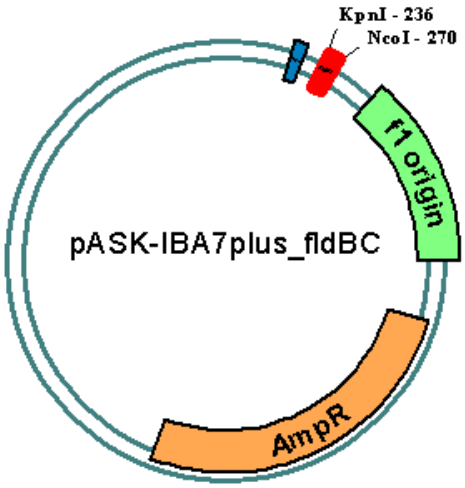



**Fig. 29** The construction of the recombinant plasmid containing *fldBC*

(f1 origin: origin of replication; AmpR: ampicillin resistance; insert: inserted gene *fldBC* in red with restriction sites of KpnI and NcoI)

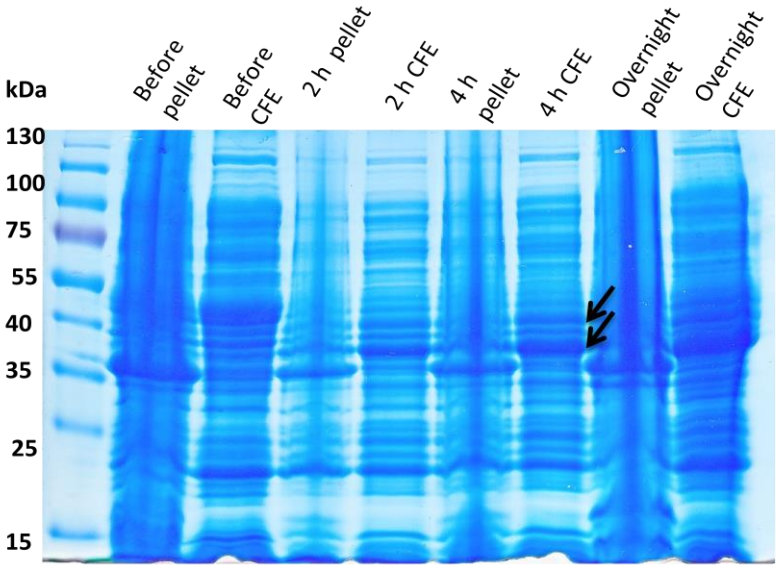



**Fig.30** The induction experiments of the gene *fldBC* in *E. coli*

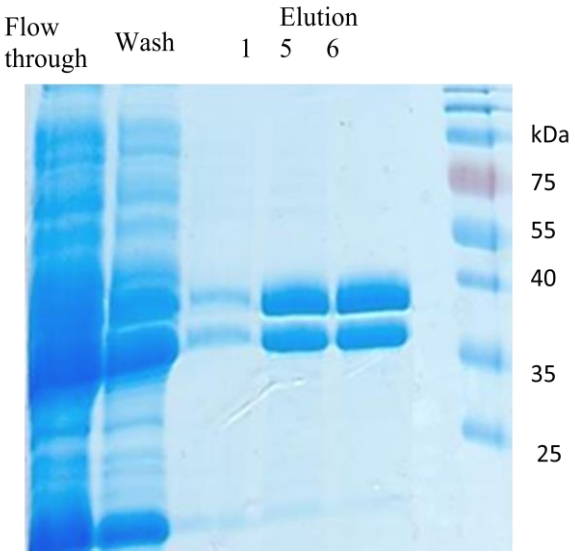
The heterologous expression of pASK-IBA7plus containing *fldBC* resulted in the production of recombinant dehydratase with an N-terminal Strep-tag. After disruption and centrifugation the dark brown cell-free supernatant, which indicated the formation of an iron sulfur cluster, was loaded on a StrepTactin column. The affinity purification was performed in an anoxic chamber. The enzyme was eluted using 3 mM desthiobiotin in Mops buffer in 2 column

volumes (Fig. 31a). The final chromatography on Superdex 200 is shown in Fig. 32, the recombinant FldBC eluted at 74 min.

Like the native dehydratase, the purified recombinant FldBC was also applied on a Native-PAGE to check the size and separation. Fig. 31b shows that the small subunit FldC could also be separated from the enzyme complex same as the native dehydratase. According to the masses of BSA the molecular mass of FldBC around 90 kDa matches to the theoretical molecular mass. The thick and blurry band around 110 kDa should be the residue of the enzyme band.

From the Michaelis-Menten curve and Lineweaver-Burk-Diagramm (Fig. 33) following parameters could be calculated by using indolelactyl-CoA as substrate: $K_m$ = 2.8 µM; $V_{max}$ = 56 U/mg. Thus the in *E. coli* produced recombinant aryllactyl-CoA dehydratase FldBC showed a higher effective activity than the native enzyme.

The accurate molecular masses of the recombinant FldBC are shown in Fig. 34 measured by MALDI-TOF. The molecular mass of FldC with 43 kDa is same as that from the purified native enzyme complex as well as the mass calculated from the gene. The theoretical molecular mass of FldB should be 49.3 kDa including 2.9 kDa of the N-terminal Strep-tag with link. The spectrum, however, exhibited two peaks, the theoretical one at 49.1 kDa and a second peak at 46.1 kDa. Hence the mass of 3 kDa was lost, which is just same as that observed with FldB from the purified native enzyme complex. Probably the subunit FldB of the recombinant dehydratase was partially modified.




**(a)** SDS-PAGE of the purification of the recombinant FldBC

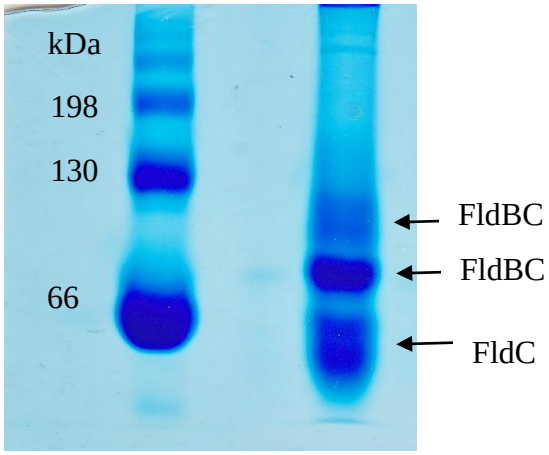



**Fig. 31 (b)** Native gel of the purification of the recombinant FldBC

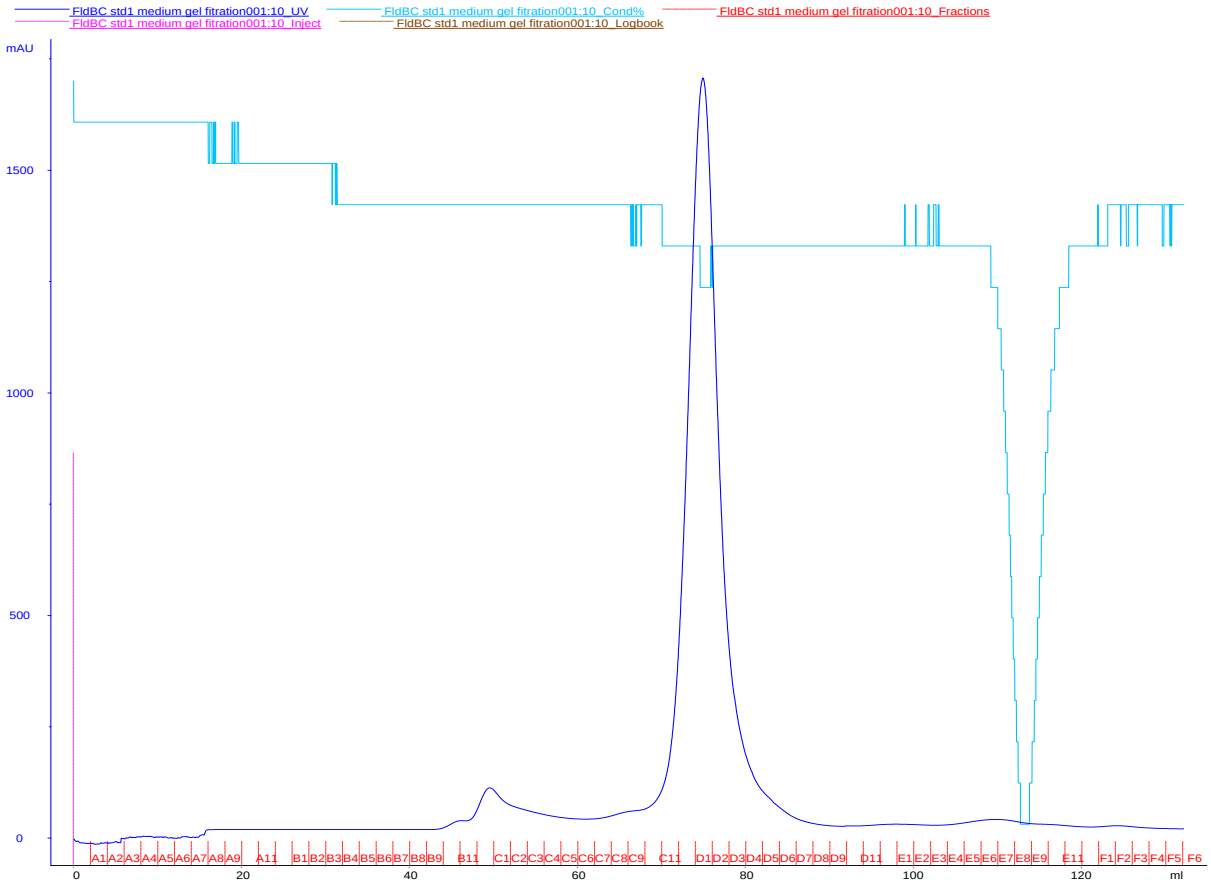



**Fig. 32** The elution chromatograph of FldBC on Superdex 200

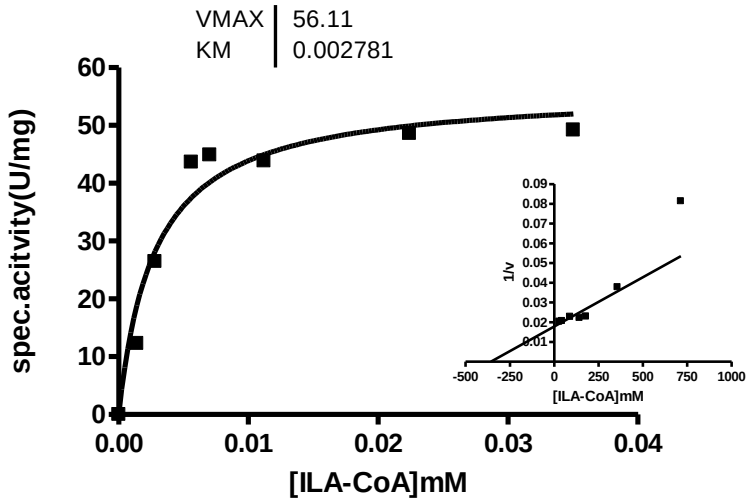



**Fig. 33** Kinetic data of the recombinant dehydratase FldBC in Michaelis-Menten graph and Lineweaver-Burk plot

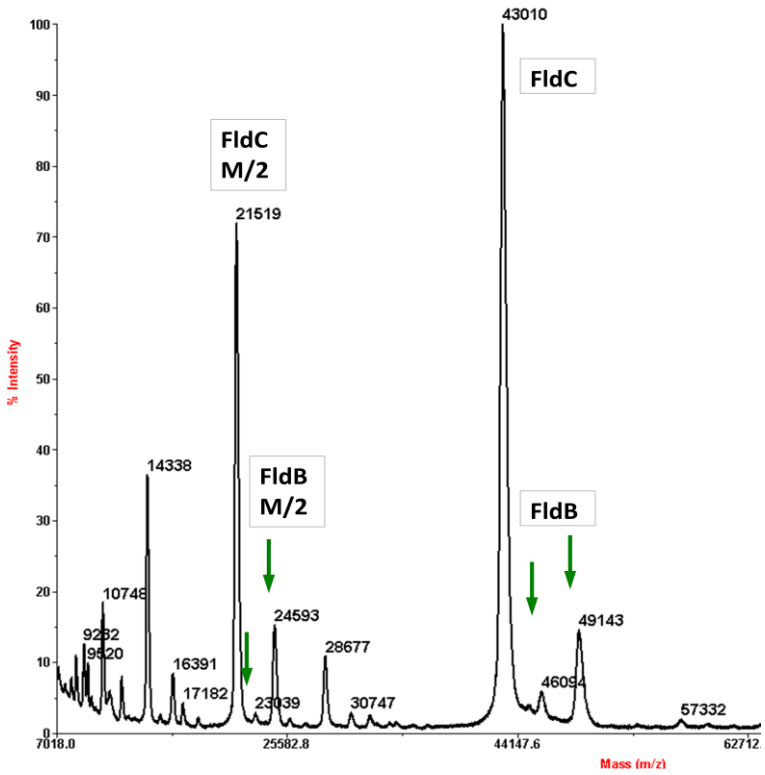



**Fig. 34** The MALDI-MS of purified recombinant FldBC

## *2.9 Fe-determination*

To confirm the amount of the [4Fe-4S] clusters in aryllactyl-CoA dehydratase, the quantities of iron in native and recombinant dehydratase were determined. According to the calibration

curve in Fig. 35 the contents of non-heme iron in native and recombinant aryllactyl-CoA dehydratases could be quantified. The native as well as recombinant enzymes contained 8~10 iron per dehydratase and confirmed the presence of two [4Fe-4S] clusters, one in each of the two subunits (Table 15).

**Table 15** Determination the concentrations of protein and [4Fe-4S] cluster according to the calibration curve

| | Iron content (mol/mol protein) |
|---|---|
| Indolelactyl-CoA dehydratase | 10 |
| Phenyllactyl-CoA dehydratase | 8.5 |
| Recombinant dehydratase | 9.8 |

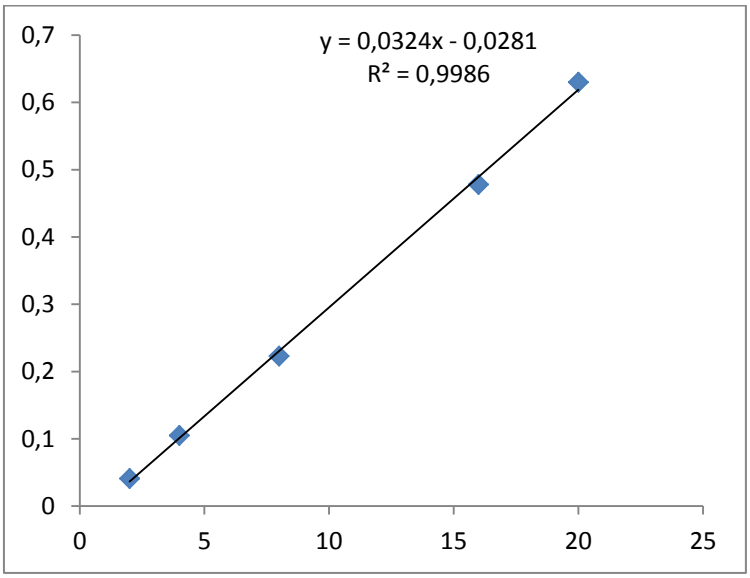
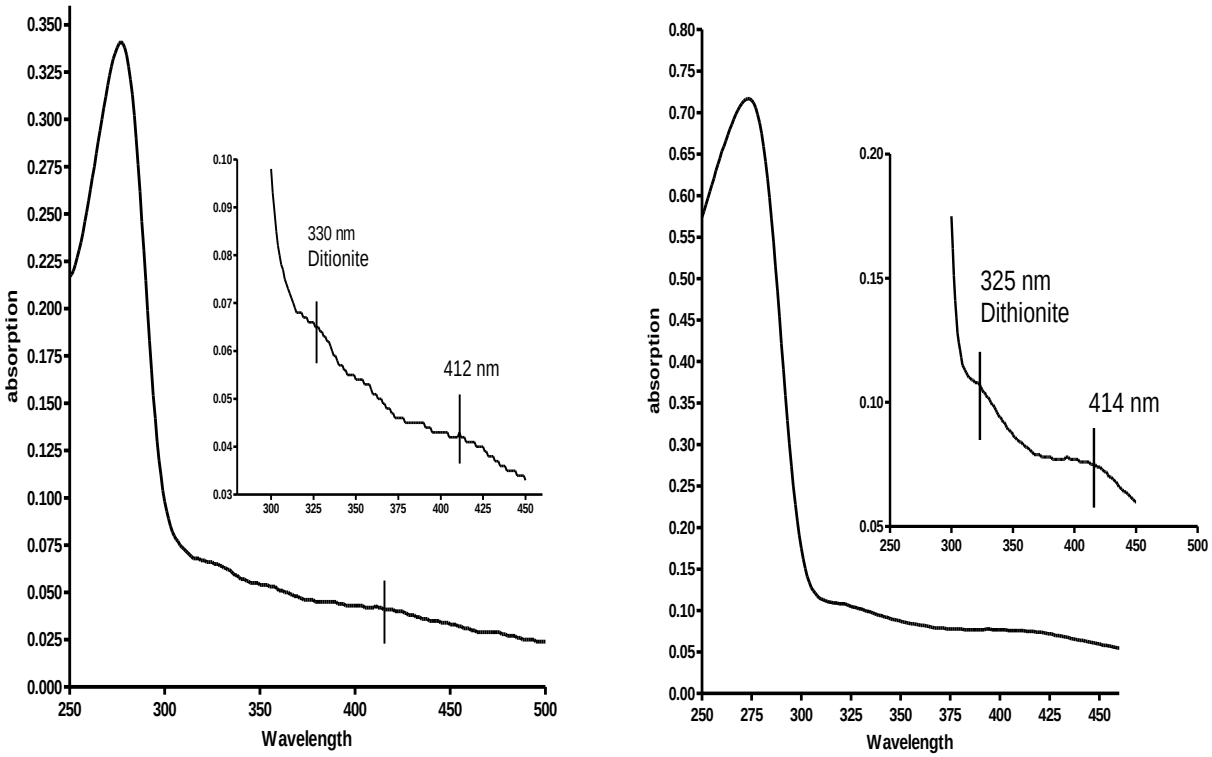



**Fig. 35** The calibration curve of non-heme Fe determination

The UV/visible spectra show the typical absorption of [4Fe-4S] at 412 nm ~ 414 nm (Fig. 36a and b). With the characteristic absorption coefficients of $\varepsilon_{280\ nm}$ = 145 mM $^{-1}$cm$^{-1}$ of aryllactatyl-CoA dehydratase FldABC at 280 nm, $\varepsilon_{280\ nm}$ = 90 mM $^{-1}$cm$^{-1}$ of recombinant FldBC (calculate tools from EnCor biotechnology Inc.) and $\varepsilon_{400\ nm}$ = 16 mM$^{-1}$cm$^{-1}$ of one [4Fe-4S] cluster at 400 nm. It confirms that the aryllactyl-CoA dehydratase contains two [4Fe-4S] clusters (Table 16).

**Table 16** Determination the concentrations of protein and [4Fe-4S] cluster by UV/visible spectra

| | protein conc. µM | [4Fe-4S] conc. µM | [4Fe-4S] content (mol/mol protein) |
|---|---|---|---|
| native dehydratase | 1.2 | 2.6 | 2.2 |
| recombinant dehydratase | 2.3 | 4.8 | 2.1 |




(a) (b)

**Fig. 36** The UV/visible spectra of purified native aryllactyl-CoA dehydratase (a) and recombinant dehydratase FldBC (b).

## *2.10 Reduction with $EuCl_2$ -DTPA complex as electron donor: Radical dehydration with an artificial electron donor $EuCl_2$*

In the introduction it has been described that the acyl-CoA dehydratatse needs one electron with a low redox-potential (< -700 mV) to derive the reaction. In the native system this electron achieves the low potential by ATP hydrolysis. Europium ($Eu^{3+/2+}$) in the presence of DTPA (diethylenetriamine-*N,N,N',N'',N''*-pentaacetate) has a reduction potential of -1.14 V at

pH 8.0 that could also reduce the dehydratase [30]. Thus 0.85 µM purified indolelactyl-CoA dehydratase was incubated with an equivalent of $EuCl_2$ and DTPA in 50 mM Tris/HCl pH 8.0 for 10 minutes at room temperature. The reactions were started by adding aryllactyl-CoA and followed at 380 nm for indolelactyl-CoA and at 310 nm for phenyllactyl-CoA. Both enzymes showed activity without the activator and extra electron source, although the activities with 140 U/mg and 6.8 mU/mg, respectively, were lower than those with activator, ATP and dithionite (10.8 U/mg; 0.9 U/mg).

## *2.11 Sequence analysis and comparison*

To find out whether there is a modification of the N- or C-terminus of the medium and small subunits of both dehydratases from Phe- or Trp-culture, the gel-bands from SDS-PAGE (Fig. 37) were digested with trypsin, endoprotease Arg-C and chymotrypsin. The obtained peptides were analyzed by MALDI-TOF peptide mass fingerprint and Nano LC-MS together with Mascot peptide databank (Supplement). Neither the N-termini nor the C-termini of the two dehydratase subunits showed deviations from the amino acid sequences predicted from the genes. The same experiments and analysis were also performed with the recombinant dehydratase subunits FldB and C. The result shows just same as that of the native dehydratase.

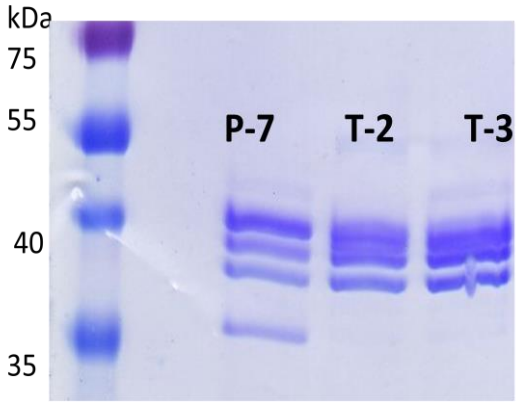



**Fig. 37** SDS-PAGE with the purified aryllactyl-CoA dehydratases from Phe- (P-7) and Trp-cultures (T-2 and T-3) for peptide sequencing

Later the native dehydratases purified from Trp- or Phe-cultures and the recombinant FldBC produced in *E. coli* were directly treated with 4-vinylpyridine to prevent disulfide formation of the cysteine residues and digested with trypsin and chymotrypsin. The obtained peptides were measured via Nano LC-MS to analyze the peptide sequences (attachment). The result of the analysis and the comparison of the amino acids sequences are shown in Table 17 and Attachment. The amino acids in red are the pieces which could be identified in the Mascot

databank with convincing score numbers over 50; the others in black had score numbers below 50 or were not detected. The peptide in blue was not detected in all three enzymes.

**Table 17** The amino acids sequences of native dehydratase and recombinant FldBC were analyzed and compared with the data-bank via peptide mass and Mascot.

```
FldB from Phe-culutr   MSDRNKEVKEKKAKHYLREITAKHYKEALEAKERGEKVGWCASNFPQEIATTLGVKVVYP
FldB from Trp-cultur   MSDRNKEVKEKKAKHYLREITAKHYKEALEAKERGEKVGWCASNFPQEIATTLGVKVVYP
FldB from recombinant MSDRNKEVKEKKAKHYLREITAKHYKEALEAKERGEKVGWCASNFPQEIATTLGVKVVYP

FldB from Phe-culutr   ENHAAAVAARGNGQNMCEHAEAMGFSNDVCGYARVNLAVMDIGHSEDQPIPMPDFVLCCN
FldB from Trp-cultur   ENHAAAVAARGNGQNMCEHAEAMGFSNDVCGYARVNLAVMDIGHSEDQPIPMPDFVLCCN
FldB from recombinant ENHAAAVAARGNGQNMCEHAEAMGFSNDVCGYARVNLAVMDIGHSEDQPIPMPDFVLCCN

FldB from Phe-culutr   NICNQMIKWYEHIAKTLDIPMILIDIPYNTENTVSQDRIKYIRAQFDDAIKQLEEITGKK
FldB from Trp-cultur   NICNQMIKWYEHIAKTLDIPMILIDIPYNTENTVSQDRIKYIRAQFDDAIKQLEEITGKK
FldB from recombinant NICNQMIKWYEHIAKTLDIPMILIDIPYNTENTVSQDRIKYIRAQFDDAIKQLEEITGKK

FldB from Phe-culutr   WDENKFEEVMKISQESAKQWLRAASYAKYKPSPFSGFDLFNHMAVAVCARGTQEAADAFK
FldB from Trp-cultur   WDENKFEEVMKISQESAKQWLRAASYAKYKPSPFSGFDLFNHMAVAVCARGTQEAADAFK
FldB from recombinant WDENKFEEVMKISQESAKQWLRAASYAKYKPSPFSGFDLFNHMAVAVCARGTQEAADAFK

FldB from Phe-culutr   MLADEYEENVKTGKSTYRGEEKQRILFEGIACWPYLRHKLTKLSEYGMNVTATVYAEAFG
FldB from Trp-cultur   MLADEYEENVKTGKSTYRGEEKQRILFEGIACWPYLRHKLTKLSEYGMNVTATVYAEAFG
FldB from recombinant MLADEYEENVKTGKSTYRGEEKQRILFEGIACWPYLRHKLTKLSEYGMNVTATVYAEAFG

FldB from Phe-culutr   VIYENMDELMAAYNKVPNSISFENALKMRLNAVTSTNTEGAVIHINRSCKLWSGFLYELA
FldB from Trp-cultur   VIYENMDELMAAYNKVPNSISFENALKMRLNAVTSTNTEGAVIHINRSCKLWSGFLYELA
FldB from recombinant VIYENMDELMAAYNKVPNSISFENALKMRLNAVTSTNTEGAVIHINRSCKLWSGFLYELA

FldB from Phe-culutr   RRLEKETGIPVVSFDGDQADPRNFSEAQYDTRIQGLNEVMVAKKEAE
FldB from Trp-cultur   RRLEKETGIPVVSFDGDQADPRNFSEAQYDTRIQGLNEVMVAKKEAE
FldB from recombinant RRLEKETGIPVVSFDGDQADPRNFSEAQYDTRIQGLNEVMVAKKEAE
```

```
FldC from Phe-culutr   MSNSDKFFNDFKDIVENPKKYIMKHMEQTGQKAIGCMPLYTPEELVLAAGMFPVGVWGSN
FldC from Try-cultur   MSNSDKFFNDFKDIVENPKKYIMKHMEQTGQKAIGCMPLYTPEELVLAAGMFPVGVWGSN
FldC from recombinant MSNSDKFFNDFKDIVENPKKYIMKHMEQTGQKAIGCMPLYTPEELVLAAGMFPVGVWGSN

FldC from Phe-culutr   TELSKAKTYFPAFICSILQTTLENALNGEYDMLSGMMITNYCDSLKCMGQNFKLTVENIE
FldC from Try-cultur   TELSKAKTYFPAFICSILQTTLENALNGEYDMLSGMMITNYCDSLKCMGQNFKLTVENIE
FldC from recombinant TELSKAKTYFPAFICSILQTTLENALNGEYDMLSGMMITNYCDSLKCMGQNFKLTVENIE

FldC from Phe-culutr   FIPVTVPQNRKMEAGKEFLKSQYKMNIEQLEKISGNKITDESLEKAIEIYDEHRKVMNDF
FldC from Try-cultur   FIPVTVPQNRKMEAGKEFLKSQYKMNIEQLEKISGNKITDESLEKAIEIYDEHRKVMNDF
FldC from recombinant FIPVTVPQNRKMEAGKEFLKSQYKMNIEQLEKISGNKITDESLEKAIEIYDEHRKVMNDF

FldC from Phe-culutr   SMLASKYPGIITPTKRNYVMKSAYYMDKKEHTEKVRQLMDEIKAIEPKPFEGKRVITTGI
FldC from Try-cultur   SMLASKYPGIITPTKRNYVMKSAYYMDKKEHTEKVRQLMDEIKAIEPKPFEGKRVITTGI
FldC from recombinant SMLASKYPGIITPTKRNYVMKSAYYMDKKEHTEKVRQLMDEIKAIEPKPFEGKRVITTGI

FldC from Phe-culutr   IADSEDLLKILEENNIAIVGDDIAHESRQYRTLTPEANTPMDRLAEQFANRECSTLYDPE
FldC from Try-cultur   IADSEDLLKILEENNIAIVGDDIAHESRQYRTLTPEANTPMDRLAEQFANRECSTLYDPE
FldC from recombinant IADSEDLLKILEENNIAIVGDDIAHESRQYRTLTPEANTPMDRLAEQFANRECSTLYDPE

FldC from Phe-culutr   KKRGQYIVEMAKERKADGIIFFMTKFCDPEEYDYPQMKKDFEEAGIPHVLIETDMQMKNY
FldC from Try-cultur   KKRGQYIVEMAKERKADGIIFFMTKFCDPEEYDYPQMKKDFEEAGIPHVLIETDMQMKNY
FldC from recombinant KKRGQYIVEMAKERKADGIIFFMTKFCDPEEYDYPQMKKDFEEAGIPHVLIETDMQMKNY

FldC from Phe-culutr   EQARTAIQAFSETL
FldC from Try-cultur   EQARTAIQAFSETL
FldC from recombinant EQARTAIQAFSETL
```

The peptide pieces in blue: HKLTKLSEYGMNVTATVYAEAFGVIYENMDELMAAYNK contains a position (in red) at which there could be an *N*-linked glycosylation at the asparagine amide nitrogen [31, 32]. This causes a different mass of the peptide that is impossible to be detected in the standard peptide mass data bank. To exclude this possibility the native enzymes were digested by chymotrypsine and then incubated with the protease inhibitor PMSF to inhibit the rest of chymotrypsine. After that the peptides were incubated with the peptide-*N*-glycosidase F (PNGase F, Biolabs) at 37 °C for 60 min to cleave between the asparagines residues and the complex oligosaccharides. After deglycosylation the asparagine N turns to asparate D and the peptide should be 0.984 dalton heavier. After the peptide finger print analysis the mass peaks were additionally fragmented again to compare the sequence with the MSMS-spectra. But in both cases there was no new peak found that matched to the modified peptide mass. Probably the peptide pieces in blue were not present.

## *2. Tryptophan transaminase, indolelactate dehydrogenase indoleacrylate reductatse and Rnf*

The activities of the three enzymes from the reductive branch of tryptophan fermentation could be determined in the cell-free extract of a culture grown on tryptophan: tryptophan transaminase with 2-oxoglutarate as second substrate 0.69 U/mg; indolelactate dehydrogenase ($NAD^+$) 0.12 U/mg; indolearcylate reductase (NADH) 0.2 U/mg. Indolelactate dehydrogenase and indoleacrylate reductase could be enriched during the purification of aryllactyl-CoA dehydatase. In the first step on the DEAE column the dehydrogenase and reductase eluted together around 0.27 M NaCl in a linear gradient from 0 to 0.6 M NaCl in 20 mM Mops pH 7.3, which was followed by the elution of dehydratase. On the next phenyl-Sepharose column the reductase eluted around 0.4 M $(NH_4)_2SO_4$ in a linear gradient from 1 M to 0.3 M $(NH_4)_2SO_4$ in 20 mM Mops pH 7.3, which directly was followed by the dehydrogenase. The molecular masses of the reductase and dehydrogenase subunits were determined by SDS-PAGE as 70 kDa and 40 kDa, respectively, the same as found previously for cinnamate reductase and phenyllactate dehydrogenase [8]. The activity of Rnf (Rhodobacter nitrogen fixation) was determined with 2 U/mg in the cell-free extract.

## 3. *Arginine fermentation in C. sporogenes, a probable oxidation partner of tryptophan in a Stickland reaction*

During purification of aryllactyl-CoA dehydratase sometimes in addition to the three bands of the dehydratase four other bands were found on SDS-PAGE (Fig. 38). With the help of MALDI-TOF MS peptide finger print, the additional bands could be identified as argininosuccinate synthase 50 kDa, ornithine carbamoyl-transferase 36 kDa, ornithine cyclodeaminase 43 kDa and selenium metabolism protein YedF 21.4 kDa. They all belong to the enzymes which participate in the fermentation of arginine via ornithine and proline by *C. sporogenes* (Fig. 39) [33].

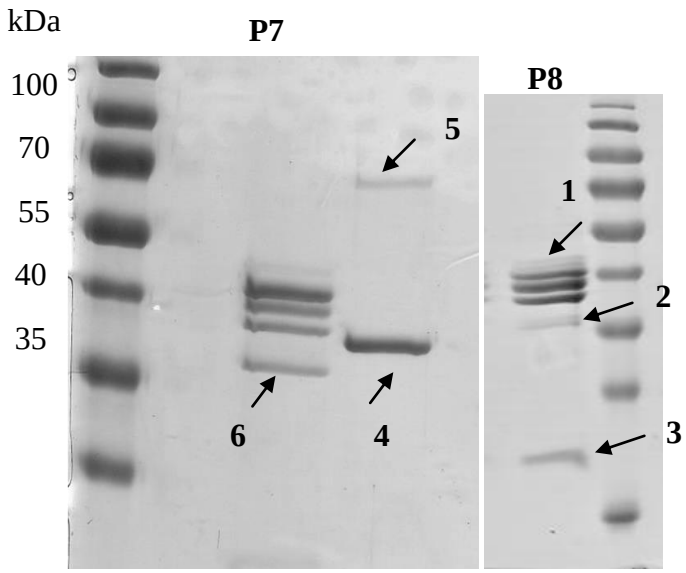



**Fig. 38** SDS-gel of the dehydratase fractions 1: argininosuccinate synthase; 2: ornithine cyclodeaminase; 3: selenium metabolism protein YedF; 4: ornithine carbamoyl-transferase; (eluted before the dehydratase from the Superdex 200 column); 5: pyruvate kinase; 6: fructose-1,6-bisphosphate aldolase.

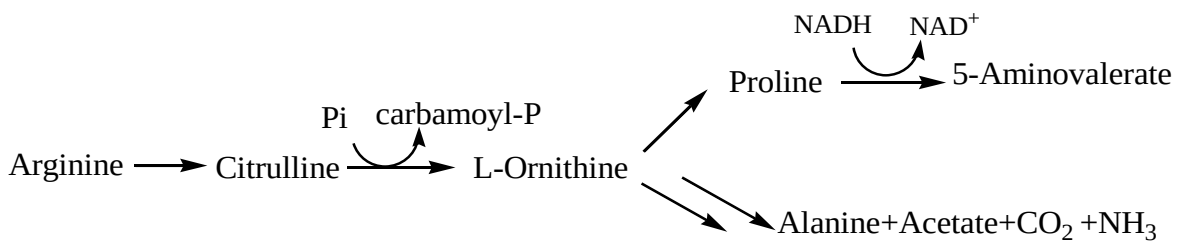



**Fig. 39** The degradation of arginine by *C. sporogenes* [33]

# *DISCUSSION*

## *1. The pathway of tryptophan fermentation in C. sporogenes*

In the classic Stickland reaction one amino acid is oxidized to a fatty acid, $CO_2$ and ammonia. In reductive branch the same or another amino is reduced to ammonia and a fatty acid of the same carbon skeleton. It was generally assumed that only in the oxidative branch energy is conserved via substrate level phosphorylation (Fig. 4). In the first part of Results it was established that tryptophan fermentation in *C. sporogenes* followed this scheme. Similar to the fermentation of phenylalanine [8], tryptophan is first transaminated to indolepyruvate, which disproportionates indoleacetate and indolpropionate. The oxidative branch conserves energy via substrate level phosphorylation from indoleacetyl-CoA. In the reductive branch indolepyruvate is reduced to indolepropionate via indolelactate and indoleacrylate. The detection of a high activity (2 U/mg) of the $Na^+$-pumping ferredoxin-NAD reductase (Rnf, [34]) in membrane preparations of *C. sporogenes* indicates, however, that also an electron transport phosphorylation occurs in this organism. The ferredoxin reduced by oxidation of indolepyruvate produces NADH for the reductive steps via Rnf, which generates an electrochemical $Na^+$-gradient for ATP synthesis. The activities of the enzymes tryptophan transaminase, indolelactate dehydrogenase, indolelactate dehydratase and indoleacrylate reductase were determined in the cell-free extract. The dehydratase complex, consisting of indolelactate CoA-transferase (FldA) and indolelactyl-CoA dehydratase (FldBC), was purified to homogeneity; the dehydrogenase and reductase were partially purified. These results confirm the new proposed pathway (Fig. 40). Recently the similar catabolic pathway of phenylalanine was also observed in the hyperthermophilic archaeon *Archaeoglobus fulgidus*. In contrast to in *C. sporogenes* this pathway is not fermentative but coupled to sulphate reduction. [35]

When in tryptophan fermenting cultures the yeast extract was increased from 0.25% to 0.50% no indoleacetate could be detected any more. Probably other amino acids of the yeast extract were oxidized faster than tryptophan. The much better growth after addition of peptone to the media confirmed this view. Further the growth experiments indicated that alanine, valine and isoleucine, which are known as the typical amino acid oxidation partners of Stickland reactions, could not significantly stimulate growth. During purification of indolelactate dehydratase other enzymes co-purified, which were identified as argininosuccinate synthase, ornithine carbamoyl-transferase, ornithine cyclodeaminase and a selenium metabolism

protein. These results suggested that *C. sporogenes* used arginine as reductant for tryptophan and was oxidized via ornithine to acetate and alanine. Part of the ornithine could be reduced to 5-aminovalerate via proline catalyzed by ornithine cyclodeaminase and the selenoenzyme proline reductase.

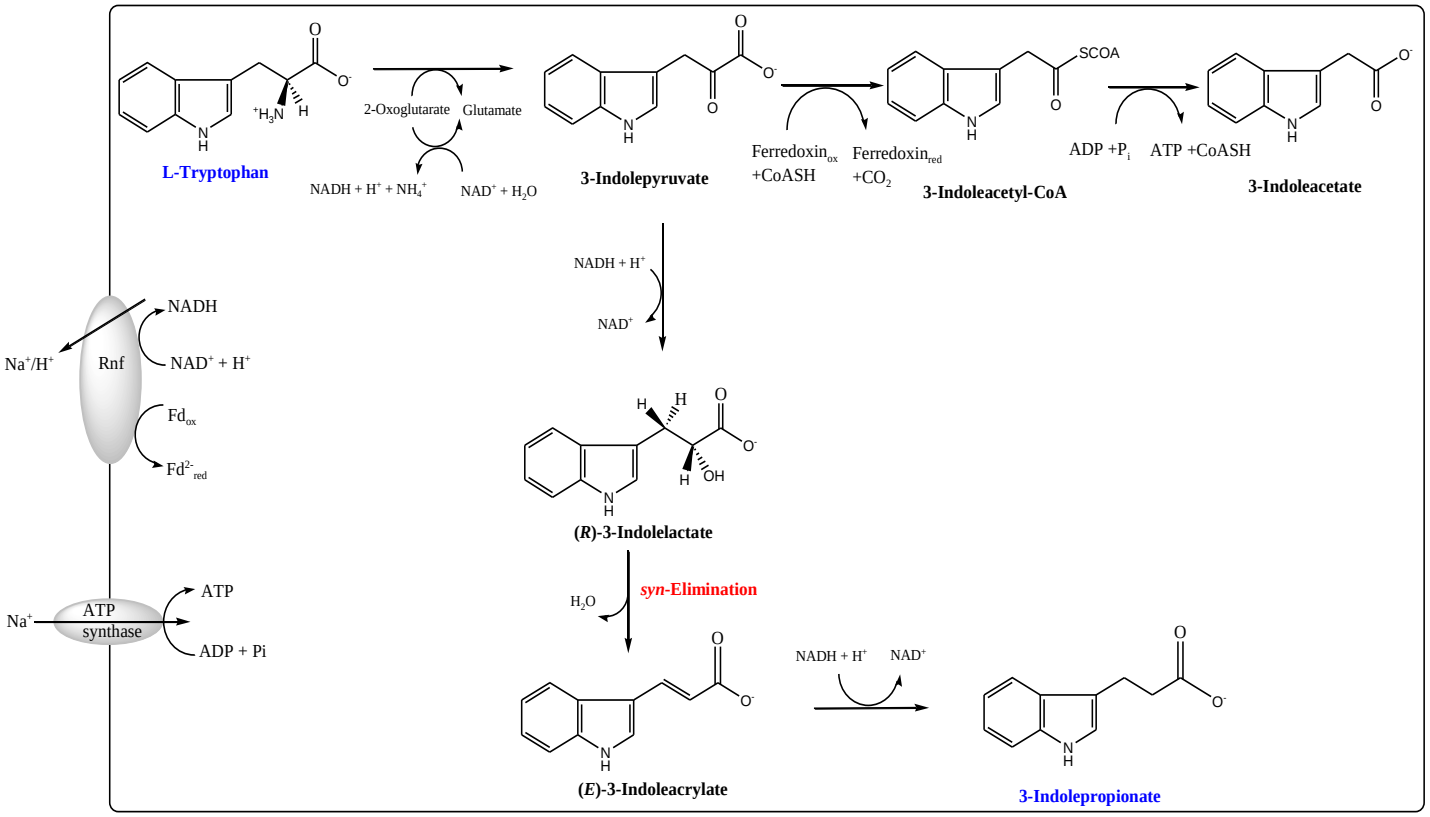



**Fig. 40** The new proposed pathway of the fermentation of tryptophan in *C. sporogenes*

In the paper of W. R. Wikoff et al. [6] it was shown that the indolepropionate of the human blood originated from the gut microflora. The authors proposed that *E. coli* and other intestinal bacteria degrade tryptophan first to indole, which is further converted to indolepropionate by *C. sporogene*s (Fig. 41). With the results of this thesis we now know that *C. sporogenes* is able to produce indolepropionate directly from tryptophan without passing through indole as intermediate (Fig. 40). A synthesis of indolepropionate from indole and perhaps acrylyl-CoA would be very unlikely.

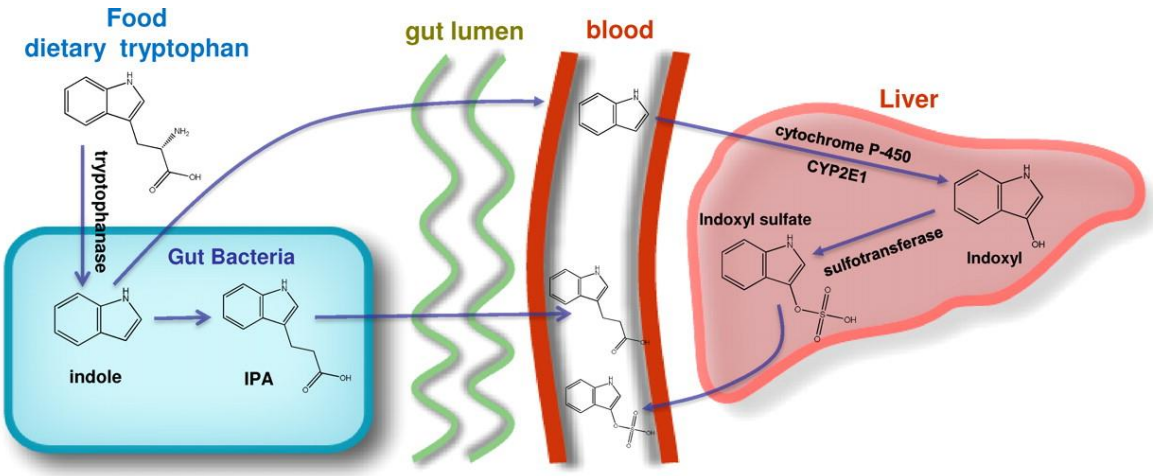
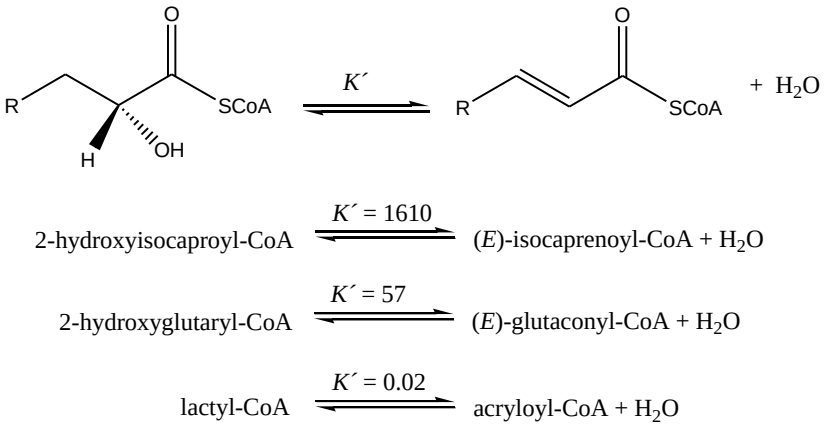



**Fig. 41** Diversity of indole-containing compounds in human serum from [6]

## 2. *Aryllactyl-CoA dehydratase complex*

The indolelactyl-CoA dehydratase was purified from the cells grew on tryptophan and the phenyllactyl-CoA dehydratase from the cells grew on phenylalanine respectively by using a newly designed purification method. At the beginning the enzyme activities and kinetics of these two dehydratases were investigated and compared. The research on the thermodynamic equilibrium constants of the radical dehydration between (*R*)-2-hydroxyacyl-CoA and 2-enoyl-CoA revealed a large effect of the substituent at the β-carbon. [36] In a combined experimental and computational approach it has been observed that in the dehydration of 2-hydroxyglutaryl-CoA the forward reaction is favored, $K'_{eq}$ = 57; with 2-hydroxyisocaproyl-CoA this effect is even more pronounced, $K'_{eq}$ = 1610. In contrast the dehydration of lactyl-CoA, which has no electron donating substituent in the β-position, exhibits an endergonic reaction with $K'_{eq}$ = 0.02 (Fig. 42a). The results of this thesis agree with that. Although the dehydration of indolelactyl-CoA to indoleacrylyl-CoA is reversible, the reaction strongly prefers to produce indoleacrylyl-CoA; the same phenomenon was observed at the dehydration of phenyllactyl-CoA to cinnamoyl-CoA, probably also same as the dehydration of *p*-hydroxyphenyllactyl-CoA to *p*-hydroxycinnamoly-CoA. Those three arylacrylyl-CoAs contain very stable extended conjugated π-systems composed of the double bonds and the carbonyl groups with the aromatic rings (Fig. 42b). The aromatic substituent at the β-position is also able to stabilize the intermediate arylallylic ketyl and arylenoxy radicals by hyperconjungation. The equilibrium constant of the reaction to indolearcylyl-CoA (*K* = 74) is

higher than the reaction to cinnamoyl-CoA ($K$ = 51), probably due to the much more extended π-system with the larger indole-ring.




(a) Equilibrium of the dehydrations of the α-hydroxyacyl-CoA [36]

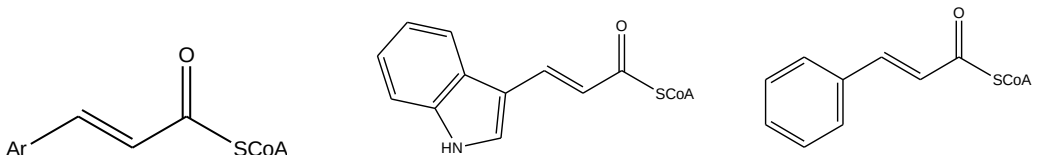



**Fig. 42** (b) Structures of aryllactyl-CoA, indolelactyl-CoA and cinnamoyl-CoA

Interestingly, the indolelactyl-CoA dehydratase and the phenyllactyl-CoA dehydratase are able to catalyze all three dehydrations of indolelactyl-CoA, phenyllactyl-CoA and 4-hydroxyphenyllactyl-CoA. But the indolelactyl-CoA dehydratase showed higher activity with indolelactyl-CoA than with phenyllactyl-CoA, whereas with phenyllactyl-CoA as substrate showed the phenyllactyl-CoA higher activity than with indolelactyl-CoA. The purified indolelactyl-CoA dehydratase and phenyllactyl-CoA dehydratase both are part of an enzyme complex containing three subunits (SDS-PAGE), which could be identified via MALDI-TOF peptide finger print as CoA-transferase (FldA) and the actual dehydratase (FldBC). According to MALDI-TOF peptide mass finger print and Nano LC-MS the peptide sequences of the native indolelactyl-CoA dehydatase complex appeared to be identical to the predicted amino acid sequences of phenyllactate dehydratase including the CoA-transferase FldA and the actual dehydratase FldBC, which were derived from *fldABC*. The recombinant two subunits of the actual dehydratase FldBC produced in *E. coli* also confirmed the amino acid sequences. The recombinant FldBC showed even higher activities with indolelactyl-CoA and also with phenyllactyl-CoA. Therefore the genes *fldABC* could be renamed as aryllactyl-CoA dehydratase encoding genes. Further experiments indicated that the dehydrogenase and the

reductase just use carboxylates as substrate and did not accept CoA-thioesters. This confirmed that the dehydration is the only step of the fermentation that requires the transformation to CoA-thioesters. There is also the gene *fldL* found upstream of the gene cluster *fldAIBC* (Fig. 12), of which the sequenced C-terminus is similar to an ORF in *C. botulinum* ATCC 3502 annotated as CoA ligase. FldL could synthesize the initial arylacrylyl-CoA. Then the family III CoA-transferase FldA transfers the CoA moiety from arylacrylyl-CoA to aryllactate and starts the cyclic reaction in Fig. 43.

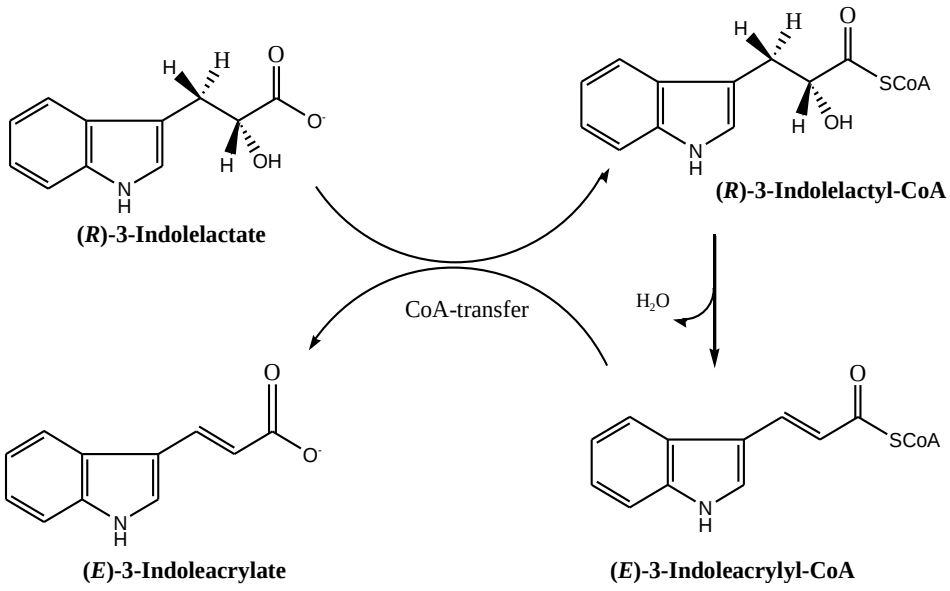



**Fig. 43** The mechanism of CoA-transfer by FldA

## *3. Modification of FldB*

The purification of the native aryllactyl-CoA dehydratases were performed via three chromatography columns. Several different columns were used but not only special for aryllactyl-CoA dehydratase purification; they were also used for other enzymes and repacked in the meantime. These led to changes of conditions and capacities of the columns, which caused different purification performances. The MALDI-TOF mass spectrometer was calibrated twice during the measurements. Therefore variations of the molecular mass determinations were observed. The average molecular mass of the medium subunit FldB was 2.0 ± 0.4 kDa lower than the calculated mass from the gene (46.2 kDa); in contrast the observed masses of FldA (46.4 kDa) and FldC (43.1 kDa) were the same as calculated. Probably FldB suffered a modification as observed earlier [23]. In this work it had been concluded that the C-terminus was removed, because the N-terminus exhibited the same sequence as derived from the gene. Interestingly, MALDI-TOF MS of FldB of the

recombinant dehydratase gave two mass peaks (46.1 kDa and 49.1 kDa). Apparently only part of FldB was modified.

Table 17 shows the amino acid sequence of FldB. The amino acids in red are confirmed by Nano LC-MS, whereas those in blue could not be identified. In contrast to the earlier suggestion [23], the N- and C-termini remained unchanged as well as the [4Fe-4S] cluster coordinating cysteine residues (Cys90, Cys123, Cys349) and the conserved glutamate (Glu61) which is hydrogen bonded to the OH-groups of the substrate and of serine, (Ser43). Probably 2.0 ± 0.4 kDa of the 4.3 kDa peptide (blue in Table 17; gray in Table 18) has been cut out.

The two subunits of aryllactyl-CoA dehydratase (FldBC) reveal sequence identities to 2-hydroxyisocaproyl-CoA deyhdratase (HadBC) of 51% and 50%, respectively (Table 18). The predicted secondary structures of FldBC (Fig.44) could be established as model based on the solved crystal structure of HadBC [21] by Pyr[2]. The part of FldB, which was not found by Nano LC-MS, is shown in the box. It consists of a loop between two α-helices.

**Table 18** Sequence alignment of FldB from *C. sporogenes* and HadB from *C. difficile*

```
C.difficile    M----KMSEKKEARVVINDLLAEQYANAFKAKEEGRPVGWSTSVFPQELAEVFDLNVLYP
C.sporogenes   MSDRNKEVKEKKAKHYLREITAKHYKEALEAKERGEKVGWCASNFPQEIATTLGVKVVYP 60
               *    *  ::*:*:  :.:: *::* :*::***.*. ***.:* ****:* .:.::*:**

C.difficile    ENQAAGVAAKKGSLELCEIAESKGYSIDLCAYARTNFGLLENGGCEALDMPAPDFLLCCN
C.sporogenes   ENHAAAVAARGNGQNMCEHAEAMGFSNDVCGYARVNLAVMDIGHSEDQPIPMPDFVLCCN 120
               **:**.***: .. ::** **: *:* *:*.***.*:.::: * .*   :* ***:****

C.difficile    NICNQVIKWYENISRELDIPLIMIDTTFNNEDEVTQSRIDYIKAQFEEAIKQLEIISGKK
C.sporogenes   NICNQMIKWYEHIAKTLDIPMILIDIPYNTENTVSQDRIKYIRAQFDDAIKQLEEITGKK 180
               *****:*****:*:: **** :*:** .:*.*: *:*.**.**:***::****** *:***

C.difficile    FDPKKFEEVMKISAENGRLWKYSMSLPADSSPSPMNGFDLFTYMAVIVCARGKKETTEAF
C.sporogenes   WDENKFEEVMKISQESAKQWLRAASY-AKYKPSPFSGFDLFNHMAVAVCARGTQEAADAF 239
               :* :********* *..: *  : *  *. .***:.*****.:*** *****.:*:::**

C.difficile    KLLIEELEDNMKTGKSSFRGEEKYRIMMEGIPCWPYIGYKMKTLAKFGVNMTGSVYPHAW
C.sporogenes   KMLADEYEENVKTGKSTYRGEEKQRILFEGIACWPYLRHKLTKLSEYGMNVTATVYAEAF 299
               *:* :* *:*:*****::***** **::***.****: :*:..*:::*:*:*.:**..*:

C.difficile    ALQYEVNDLDGMAVAYSTMFNNVNLDRMTKYRVDSLVEGKCDGAFYHMNRSCKLMSLIQY
C.sporogenes   GVIYE--NMDELMAAYNKVPNSISFENALKMRLNAVTSTNTEGAVIHINRSCKLWSGFLY 357
               .: **  ::* : .**..: *.:.::.  * *:::::.. : :**. *:****** * : *

C.difficile    EMQRRAAEETGLPYAGFDGDQADPRAFTNAQFETRIQGLVEVMEERKKLNRGEI
C.sporogenes   ELARRLEKETGIPVVSFDGDQADPRNFSEAQYDTRIQGLNEVMVAKKEAE----       407
               *: **  :***:* ..********* *::**::****** ***  :*: :
```

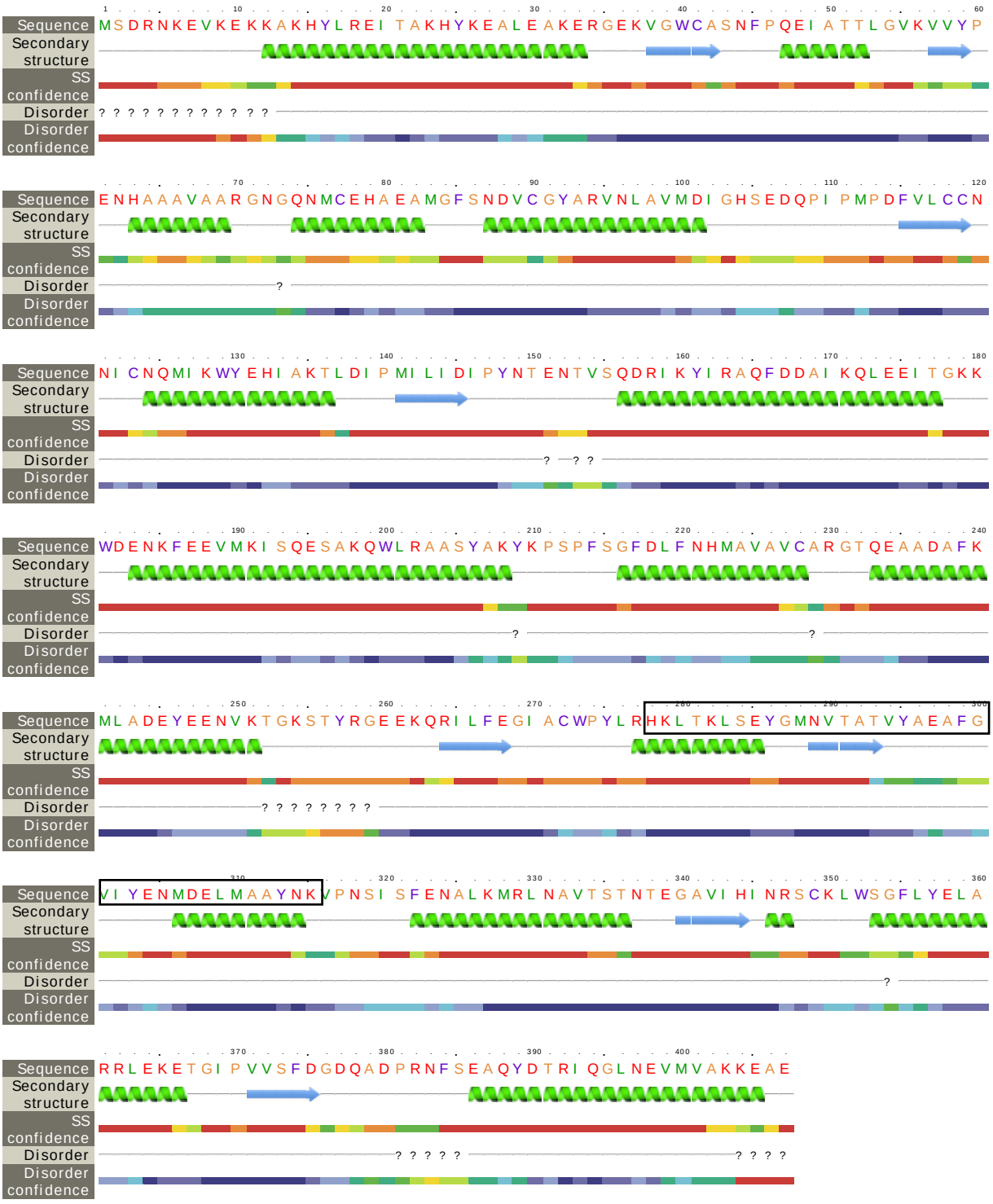



**Fig. 44** Predicted secondary structure of FldB by Pyr[2].

Based on the crystal structure of HadB (Fig. 45a), the 3D structure of FldB (Fig. 45b) could also be predicted. The part of FldB, which was not detected by Nano LC-MS, and the corresponding part of HadB are in yellow. In the structure of HadB, the loop locates opposite to the substrate binding site, actually direct opposite to the carbon-chain of the substrate, 2-hydroxyisocaproyl-CoA. Phenyllactyl-CoA, 4-hydroxyphenyllactyl-CoA and indolelactyl-CoA have a more extended carbon-chain at C4, especially the indole double ring. From the structure of HadB modeled with indolelactyl-CoA as substrate, Tyr290 and Trp294 in red in Table 18 would have to move away to make space for the indole double ring (personal discussion with Dr. B. Martin, Humboldt University Berlin). It seems likely that a posttranslational modification is necessary to make enough space to bind the substrate aryllactyl-CoA. Actually also with 2-hydroxyisocaproyl-CoA indolelactyl-CoA dehydratase as well as recombinant FldBC showed activity, albeit very low, 0.01 U/mg and 0.14 U/mg, respectively.


**Fig. 45a** The crystal structure of 2-hydroxyisocaproyl-CoA dehydratase HadBC with substrate (HadB, magenta; HadC, blue). The green circle marks the 2-hydroxyisocaproyl

moiety of the CoA-thioester near the [4Fe-4S] cluster. The corresponding peptide, which was not detected in FldB, is shown in yellow.

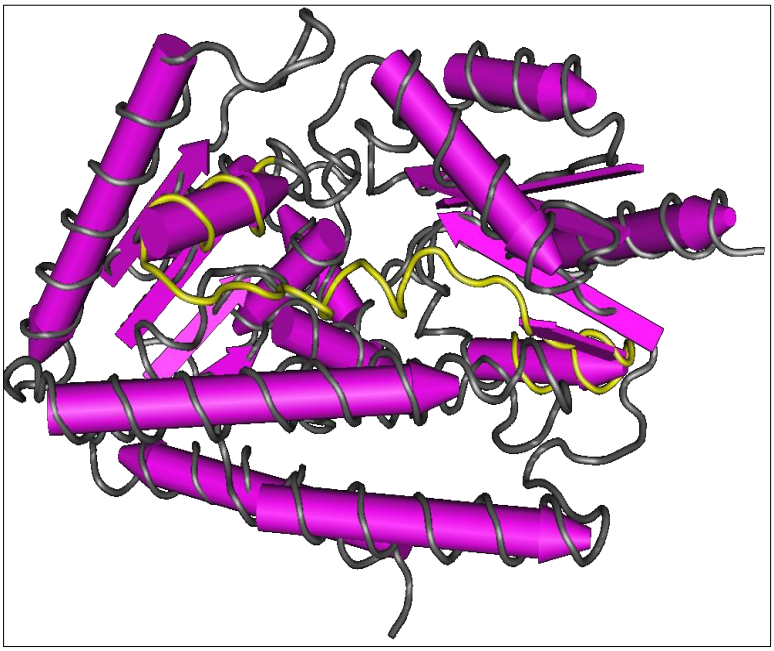


**Fig. 45b** Predicted 3D structure of FldB via Pyr[2]. The missing 4.3 kDa peptide is shown in yellow.
(Due to a printing error two β-strands are in bright red, whereas the others and the α-helices are in magenta)

Despite many sequence analyses with different proteases and methods, the peptide HKLTKLSEYGMNVTATVYAEAFGVIYENMDELMAAYN comprising 37 amino acids could not be detected in FldB. A modification at the putative glycosylation site **N**VT [37] was also excluded. The 4.3 kDa molecular mass of this peptide does not match to the mass difference between experimental and theoretical data of 2.0 ± 0.4 kDa. Furthermore a removal of the whole peptide would have led to a very big conformational change of FldB. Therefore we assume that a peptide of about 20 amino acids was cut out. From the predicted model of FldB it is unlikely that the 20 amino acid peptide between the two α-helices was cut off, because recombining of the two ends would also cause a restructuring of the whole FldB (Fig. 46a). In Fig. 46b there is a hypothesized possibility of modification. The C-terminal half of this peptide including Tyr295 and Phe299 (marked red in Table 18 and Fig. 46a and b) could be cut out. The resulting two terminals are near to each other to be connected again without a big structural change. Modeling studies showed that Tyr295 and Phe299 would interfere with the bulky substrates phenyllactyl-CoA or indolelactyl-CoA.

The results of the kinetic research show that the highest specific activities of the aryllactyl-CoA dehydratase were obtained with that aryllactyl-CoA as substrate which was derived from

the amino acid used for growth. The different amino acids could induce modifications at different positions. To fit the specific aryllactyl-CoA the purified native aryllactate deyhdratase complexes from tryptophan or phenylalanine cultures as well as the recombinant aryllactyl-CoA dehydratase FldBC are crystallizing in collaboration with Dr. Martins at the Humboldt University of Berlin. Hopefully, crystallography can determine the exact posttranslational modification, which could have occurred by protein splicing [38]. However, in all protein splicings, which are found until now, internal peptides of 134-600 amino acids, called inteins, are removed. Furthermore, the conserved amino acid residues that participate in the splicing process are missing [39, 40]. Thus this protein modification described in this thesis could be a hitherto unrecognized process.

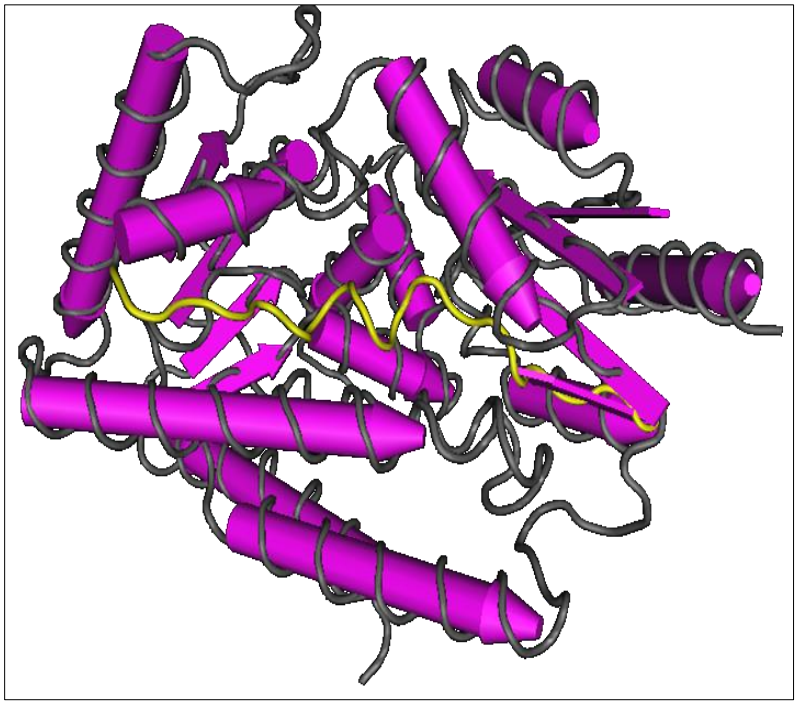


**Fig. 46a** Predicted structure of FldB. The peptide between the two helices is shown in yellow.

HKLTKLSEYGMNVTATVYAEAFGVIYENMDELMAAYN-2.2 kDa

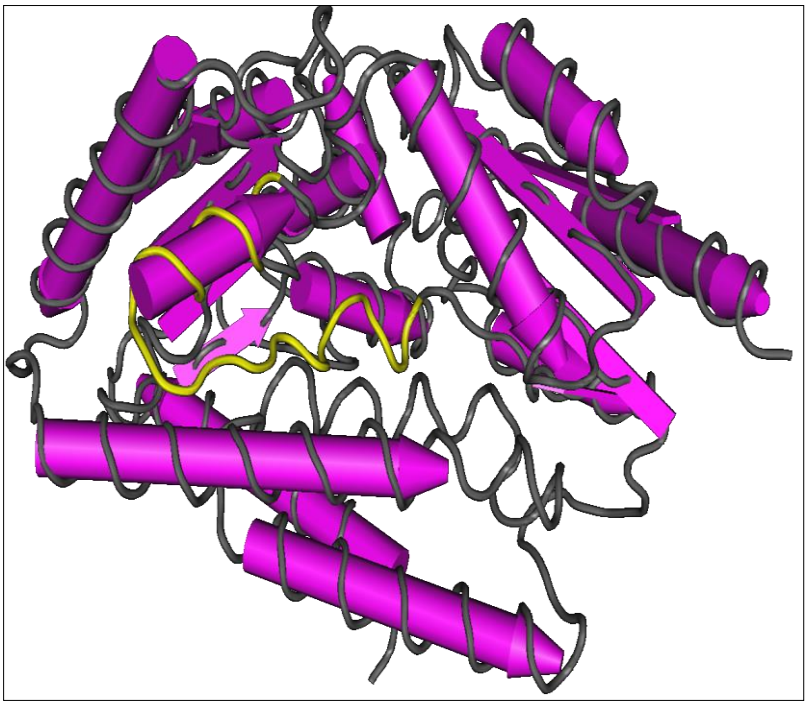


**Fig. 46b** Predicted structure of FldB. The peptide proposed to be cut out is shown in yellow.

HKLTKLSEYGMNVTATVYAEAFGVIYENMDELMAAYN-2.3 kDa

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# *SUPPLEMENT*

## *1. The dehydratase from the tryptophan-culture*

### Protein View of the medium subunit FldB of dehydratase from Trp-culture

Match to: **R-phenyllactate-medium-su** Score: **4333**
**dehydratase medium subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **46208**; Calculated pI value: **5.75**
NCBI BLAST search of R-phenyllactate-medium-su against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **76%**

Matched peptides shown in **Bold**

```
     1 MSDRNKEVKE KKAKHYLREI TAKHYKEALE AKERGEKVGW CASNFPQEIA
    51 TTLGVKVVYP ENHAAAVAAR GNGQNMCEHA EAMGFSNDVC GYARVNLAVM
   101 DIGHSEDQPI PMPDFVLCCN NICNQMIKWY EHIAKTLDIP MILIDIPYNT
   151 ENTVSQDRIK YIRAQFDDAI KQLEEITGKK WDENKFEEVM KISQESAKQW
   201 LRAASYAKYK PSPFSGFDLF NHMAVAVCAR GTQEAADAFK MLADEYEENV
   251 KTGKSTYRGE EKQRILFEGI ACWPYLRHKL TKLSEYGMNV TATVYAEAFG
   301 VIYENMDELM AAYNKVPNSI SFENALKMRL NAVTSTNTEG AVIHINRSCK
   351 LWSGFLYELA RRLEKETGIP VVSFDGDQAD PRNFSEAQYD TRIQGLNEVM
   401 VAKKEAE
```

| Start - End | Observed | Mr(expt) | Mr(calc) | Delta | Miss | Sequence |
|---|---|---|---|---|---|---|
| 15 - 21 | 931.50 | 930.49 | 930.49 | -0.00 | 0 | K.HYLREIT.A (Ions score 36) |
| 15 - 23 | 1130.64 | 1129.63 | 1129.62 | 0.01 | 0 | K.HYLREITAK.H (Ions score 47) |
| 15 - 32 | 2200.21 | 2199.21 | 2199.18 | 0.03 | 0 | K.HYLREITAKHYKEALEAK.E (Ions score 92) |
| 15 - 37 | 2799.53 | 2798.52 | 2798.48 | 0.04 | 0 | K.HYLREITAKHYKEALEAKERGEK.V (Ions score 16) |
| 17 - 25 | 1130.61 | 1129.60 | 1129.62 | -0.02 | 0 | Y.LREITAKHY.K (Ions score 13) |
| 19 - 34 | 1916.00 | 1914.99 | 1915.02 | -0.02 | 0 | R.EITAKHYKEALEAKER.G (Ions score 116) |
| 19 - 37 | 2230.20 | 2229.19 | 2229.18 | 0.01 | 0 | R.EITAKHYKEALEAKERGEK.V (Ions score 105) |
| 24 - 32 | 1088.56 | 1087.56 | 1087.57 | -0.01 | 0 | K.HYKEALEAK.E (Ions score 43) |
| 24 - 32 | 1088.59 | 1087.58 | 1087.57 | 0.02 | 0 | K.HYKEALEAK.E (Ions score 78) |
| 24 - 34 | 1373.71 | 1372.71 | 1372.71 | -0.00 | 0 | K.HYKEALEAKER.G (Ions score 99) |
| 24 - 34 | 1373.72 | 1372.72 | 1372.71 | 0.01 | 0 | K.HYKEALEAKER.G (Ions score 89) |
| 24 - 37 | 1687.87 | 1686.86 | 1686.87 | -0.01 | 0 | K.HYKEALEAKERGEK.V (Ions score 115) |
| 24 - 37 | 1687.91 | 1686.90 | 1686.87 | 0.03 | 0 | K.HYKEALEAKERGEK.V (Ions score 108) |
| 24 - 40 | 2030.07 | 2029.06 | 2029.04 | 0.02 | 0 | K.HYKEALEAKERGEKVGW.C (Ions score 122) |
| 24 - 56 | 3795.04 | 3794.04 | 3793.92 | 0.11 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 45) |
| 26 - 37 | 1387.73 | 1386.73 | 1386.75 | -0.02 | 0 | Y.KEALEAKERGEK.V (Ions score 33) |
| 26 - 40 | 1729.94 | 1728.94 | 1728.92 | 0.02 | 0 | Y.KEALEAKERGEKVGW.C (Ions score 92) |
| 27 - 34 | 945.49 | 944.48 | 944.49 | -0.01 | 0 | K.EALEAKER.G (Ions score 16) |
| 27 - 34 | 945.49 | 944.49 | 944.49 | -0.01 | 0 | K.EALEAKER.G (Ions score 35) |
| 27 - 40 | 1601.85 | 1600.84 | 1600.82 | 0.02 | 0 | K.EALEAKERGEKVGW.C (Ions score 62) |
| 27 - 56 | 3366.82 | 3365.81 | 3365.71 | 0.10 | 0 | K.EALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 14) |
| 33 - 56 | 2725.42 | 2724.41 | 2724.37 | 0.04 | 0 | K.ERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 59) |
| 35 - 56 | 2440.21 | 2439.20 | 2439.23 | -0.02 | 0 | R.GEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 99) |
| 38 - 56 | 2126.06 | 2125.05 | 2125.07 | -0.02 | 0 | K.VGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 144) |
| 38 - 56 | 2126.08 | 2125.08 | 2125.07 | 0.01 | 0 | K.VGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 16) |
| 38 - 70 | 3574.80 | 3573.80 | 3573.82 | -0.02 | 0 | K.VGWCASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 174) |
| 41 - 53 | 1499.73 | 1498.72 | 1498.71 | 0.01 | 0 | W.CASNFPQEIATTL.G S-pyridylethyl (C) (Ions score 82) |
| 46 - 53 | 872.48 | 871.47 | 871.47 | 0.01 | 0 | F.PQEIATTL.G (Ions score 69) |
| 54 - 70 | 1751.96 | 1750.96 | 1750.95 | 0.01 | 0 | L.GVKVVYPENHAAAVAAR.G (Ions score 136) |
| 56 - 70 | 1595.85 | 1594.84 | 1594.86 | -0.02 | 0 | V.KVVYPENHAAAVAAR.G (Ions score 106) |
| 56 - 70 | 1595.85 | 1594.85 | 1594.86 | -0.01 | 0 | V.KVVYPENHAAAVAAR.G (Ions score 62) |
| 57 - 66 | 1070.50 | 1069.49 | 1069.52 | -0.03 | 0 | K.VVYPENHAAA.V (Ions score 59) |
| 57 - 68 | 1240.64 | 1239.63 | 1239.62 | 0.01 | 0 | K.VVYPENHAAAVA.A (Ions score 50) |
| 57 - 70 | 1467.76 | 1466.75 | 1466.76 | -0.01 | 0 | K.VVYPENHAAAVAAR.G (Ions score 89) |
| 57 - 70 | 1467.77 | 1466.76 | 1466.76 | -0.00 | 0 | K.VVYPENHAAAVAAR.G (Ions score 15) |
| 57 - 70 | 1467.82 | 1466.81 | 1466.76 | 0.05 | 0 | K.VVYPENHAAAVAAR.G (Ions score 121) |
| 57 - 71 | 1524.79 | 1523.78 | 1523.78 | -0.00 | 0 | K.VVYPENHAAAVAARG.N (Ions score 42) |
| 57 - 83 | 2945.33 | 2944.32 | 2944.32 | 0.01 | 0 | K.VVYPENHAAAVAARGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 42) |
| 58 - 70 | 1368.67 | 1367.66 | 1367.69 | -0.03 | 0 | V.VYPENHAAAVAAR.G (Ions score 74) |
| 58 - 70 | 1368.73 | 1367.72 | 1367.69 | 0.03 | 0 | V.VYPENHAAAVAAR.G (Ions score 86) |
| 69 - 83 | 1723.67 | 1722.67 | 1722.70 | -0.04 | 0 | A.ARGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 75) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 70 - 83 | 1652.64 | 1651.64 | 1651.67 | -0.03 | 0 | A.RGNGQNMCEHAEAM.G | S-pyridylethyl (C) (Ions score 58) |
| 71 - 81 | 1294.47 | 1293.46 | 1293.49 | -0.02 | 0 | R.GNGQNMCEHAE.A | S-pyridylethyl (C) (Ions score 43) |
| 71 - 82 | 1365.51 | 1364.51 | 1364.52 | -0.02 | 0 | R.GNGQNMCEHAEA.M | S-pyridylethyl (C) (Ions score 47) |
| 71 - 83 | 1496.57 | 1495.56 | 1495.56 | -0.00 | 0 | R.GNGQNMCEHAEAM.G | S-pyridylethyl (C) (Ions score 78) |
| 71 - 83 | 1497.51 | 1496.50 | 1496.55 | -0.04 | 0 | R.GNGQNMCEHAEAM.G | Deamidation (NQ); S-pyridylethyl (C) (Ions score 20) |
| 71 - 83 | 1512.55 | 1511.54 | 1511.56 | -0.01 | 0 | R.GNGQNMCEHAEAM.G | Oxidation (M); S-pyridylethyl (C) (Ions score 46) |
| 71 - 85 | 1700.67 | 1699.66 | 1699.65 | 0.01 | 0 | R.GNGQNMCEHAEAMGF.S | S-pyridylethyl (C) (Ions score 77) |
| 71 - 85 | 1701.61 | 1700.61 | 1700.64 | -0.03 | 0 | R.GNGQNMCEHAEAMGF.S | Deamidation (NQ); S-pyridylethyl (C) (Ions score 62) |
| 71 - 85 | 1716.67 | 1715.66 | 1715.65 | 0.01 | 0 | R.GNGQNMCEHAEAMGF.S | Oxidation (M); S-pyridylethyl (C) (Ions score 57) |
| 71 - 92 | 2349.88 | 2348.87 | 2348.86 | 0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGY.A | Oxidation (M) (Ions score 14) |
| 71 - 94 | 2666.10 | 2665.09 | 2665.06 | 0.03 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 86) |
| 71 - 94 | 2667.06 | 2666.05 | 2666.04 | 0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); S-pyridylethyl (C) (Ions score 31) |
| 71 - 94 | 2668.02 | 2667.01 | 2667.02 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 Deamidation (NQ); S-pyridylethyl (C) (Ions score 61) |
| 71 - 94 | 2771.11 | 2770.11 | 2770.11 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 121) |
| 71 - 94 | 2771.21 | 2770.20 | 2770.11 | 0.09 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 161) |
| 71 - 94 | 2772.16 | 2771.15 | 2771.10 | 0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); 2 S-pyridylethyl (C) (Ions score 60) |
| 71 - 94 | 2787.17 | 2786.17 | 2786.11 | 0.06 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 98) |
| 71 - 94 | 2803.16 | 2802.15 | 2802.10 | 0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 Oxidation (M); 2 S-pyridylethyl (C) (Ions score 33) |
| 73 - 94 | 2600.10 | 2599.09 | 2599.05 | 0.04 | 0 | N.GQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 10) |
| 84 - 92 | 1066.41 | 1065.40 | 1065.42 | -0.02 | 0 | M.GFSNDVCGY.A | S-pyridylethyl (C) (Ions score 47) |
| 84 - 94 | 1293.57 | 1292.56 | 1292.56 | -0.00 | 0 | M.GFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 21) |
| 86 - 92 | 862.31 | 861.31 | 861.33 | -0.03 | 0 | F.SNDVCGY.A | S-pyridylethyl (C) (Ions score 39) |
| 129 - 135 | 946.47 | 945.46 | 945.47 | -0.01 | 0 | K.WYEHIAK.T | (Ions score 31) |
| 136 - 158 | 2661.32 | 2660.31 | 2660.34 | -0.03 | 0 | K.TLDIPMILIDIPYNTENTVSQDR.I | (Ions score 86) |
| 149 - 158 | 1163.52 | 1162.51 | 1162.52 | -0.01 | 0 | Y.NTENTVSQDR.I | (Ions score 68) |
| 149 - 160 | 1404.70 | 1403.69 | 1403.70 | -0.01 | 0 | Y.NTENTVSQDRIK.Y | (Ions score 54) |
| 161 - 179 | 2238.18 | 2237.17 | 2237.17 | 0.00 | 0 | K.YIRAQFDDAIKQLEEITGK.K | (Ions score 44) |
| 161 - 180 | 2366.30 | 2365.29 | 2365.26 | 0.03 | 0 | K.YIRAQFDDAIKQLEEITGKK.W | (Ions score 88) |
| 164 - 171 | 907.44 | 906.43 | 906.44 | -0.01 | 0 | R.AQFDDAIK.Q | (Ions score 65) |
| 164 - 171 | 907.45 | 906.44 | 906.44 | -0.00 | 0 | R.AQFDDAIK.Q | (Ions score 72) |
| 164 - 179 | 1805.94 | 1804.94 | 1804.92 | 0.02 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 123) |
| 164 - 179 | 1805.94 | 1804.94 | 1804.92 | 0.02 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 108) |
| 164 - 180 | 1934.02 | 1933.01 | 1933.02 | -0.00 | 0 | R.AQFDDAIKQLEEITGKK.W | (Ions score 46) |
| 164 - 180 | 1934.08 | 1933.07 | 1933.02 | 0.05 | 0 | R.AQFDDAIKQLEEITGKK.W | (Ions score 127) |
| 164 - 181 | 2120.11 | 2119.10 | 2119.09 | 0.00 | 0 | R.AQFDDAIKQLEEITGKKW.D | (Ions score 78) |
| 164 - 191 | 3369.66 | 3368.65 | 3368.66 | -0.01 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 116) |
| 164 - 191 | 3369.69 | 3368.68 | 3368.66 | 0.02 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 135) |
| 172 - 180 | 1045.60 | 1044.59 | 1044.58 | 0.01 | 0 | K.QLEEITGKK.W | (Ions score 57) |
| 180 - 191 | 1582.74 | 1581.74 | 1581.75 | -0.01 | 0 | K.KWDENKFEEVMK.I | (Ions score 102) |
| 180 - 191 | 1582.78 | 1581.78 | 1581.75 | 0.03 | 0 | K.KWDENKFEEVMK.I | (Ions score 103) |
| 180 - 191 | 1598.77 | 1597.76 | 1597.74 | 0.01 | 0 | K.KWDENKFEEVMK.I | Oxidation (M) (Ions score 53) |
| 180 - 198 | 2326.15 | 2325.14 | 2325.13 | 0.01 | 0 | K.KWDENKFEEVMKISQESAK.Q | (Ions score 58) |
| 180 - 202 | 2909.43 | 2908.42 | 2908.45 | -0.03 | 0 | K.KWDENKFEEVMKISQESAKQWLR.A | (Ions score 156) |
| 181 - 191 | 1454.64 | 1453.63 | 1453.65 | -0.02 | 0 | K.WDENKFEEVMK.I | (Ions score 67) |
| 181 - 191 | 1454.67 | 1453.67 | 1453.65 | 0.01 | 0 | K.WDENKFEEVMK.I | (Ions score 68) |
| 191 - 200 | 1204.61 | 1203.60 | 1203.62 | -0.02 | 0 | M.KISQESAKQW.L | (Ions score 37) |
| 192 - 200 | 1076.55 | 1075.54 | 1075.53 | 0.01 | 0 | K.ISQESAKQW.L | (Ions score 32) |
| 209 - 230 | 2562.22 | 2561.21 | 2561.23 | -0.02 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G | S-pyridylethyl (C) (Ions score 136) |
| 215 - 230 | 1842.92 | 1841.92 | 1841.87 | 0.05 | 0 | F.SGFDLFNHMAVAVCAR.G | S-pyridylethyl (C) (Ions score 47) |
| 231 - 240 | 1037.46 | 1036.46 | 1036.48 | -0.03 | 0 | R.GTQEAADAFK.M | (Ions score 68) |
| 231 - 240 | 1037.49 | 1036.49 | 1036.48 | 0.01 | 0 | R.GTQEAADAFK.M | (Ions score 75) |
| 231 - 254 | 2645.24 | 2644.23 | 2644.23 | -0.00 | 0 | R.GTQEAADAFKMLADEYEENVKTGK.S | (Ions score 64) |
| 241 - 251 | 1340.61 | 1339.60 | 1339.60 | 0.01 | 0 | K.MLADEYEENVK.T | (Ions score 79) |
| 241 - 251 | 1340.61 | 1339.61 | 1339.60 | 0.01 | 0 | K.MLADEYEENVK.T | (Ions score 78) |
| 241 - 254 | 1626.77 | 1625.76 | 1625.76 | -0.00 | 0 | K.MLADEYEENVKTGK.S | (Ions score 110) |
| 241 - 254 | 1626.78 | 1625.77 | 1625.76 | 0.01 | 0 | K.MLADEYEENVKTGK.S | (Ions score 102) |
| 241 - 262 | 2577.22 | 2576.22 | 2576.21 | 0.01 | 0 | K.MLADEYEENVKTGKSTYRGEEK.Q | (Ions score 90) |
| 241 - 264 | 2861.39 | 2860.39 | 2860.37 | 0.02 | 0 | K.MLADEYEENVKTGKSTYRGEEKQR.I | (Ions score 100) |
| 255 - 262 | 969.46 | 968.45 | 968.46 | -0.00 | 0 | K.STYRGEEK.Q | (Ions score 24) |
| 255 - 264 | 1253.63 | 1252.62 | 1252.62 | 0.01 | 0 | K.STYRGEEKQR.I | (Ions score 58) |
| 258 - 264 | 902.47 | 901.46 | 901.47 | -0.01 | 0 | Y.RGEEKQR.I | (Ions score 22) |
| 265 - 277 | 1580.80 | 1579.79 | 1579.82 | -0.03 | 0 | R.ILFEGIACWPYLR.H | (Ions score 51) |
| 265 - 277 | 1685.88 | 1684.88 | 1684.88 | -0.00 | 0 | R.ILFEGIACWPYLR.H | S-pyridylethyl (C) (Ions score 22) |
| 265 - 277 | 1685.90 | 1684.90 | 1684.88 | 0.02 | 0 | R.ILFEGIACWPYLR.H | S-pyridylethyl (C) (Ions score 81) |
| 316 - 327 | 1318.70 | 1317.70 | 1317.69 | 0.00 | 0 | K.VPNSISFENALK.M | (Ions score 100) |
| 319 - 327 | 1008.53 | 1007.52 | 1007.53 | -0.01 | 0 | N.SISFENALK.M | (Ions score 39) |
| 328 - 347 | 2197.19 | 2196.18 | 2196.14 | 0.03 | 0 | K.MRLNAVTSTNTEGAVIHINR.S | (Ions score 87) |
| 330 - 347 | 1910.01 | 1909.01 | 1909.00 | 0.00 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 104) |
| 330 - 347 | 1910.04 | 1909.03 | 1909.00 | 0.03 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 178) |
| 330 - 347 | 1910.05 | 1909.04 | 1909.00 | 0.04 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 159) |
| 331 - 347 | 1796.95 | 1795.94 | 1795.92 | 0.02 | 0 | L.NAVTSTNTEGAVIHINR.S | (Ions score 55) |
| 351 - 361 | 1354.71 | 1353.71 | 1353.71 | -0.00 | 0 | K.LWSGFLYELAR.R | (Ions score 105) |
| 351 - 361 | 1354.74 | 1353.73 | 1353.71 | 0.02 | 0 | K.LWSGFLYELAR.R | (Ions score 105) |
| 362 - 374 | 1474.82 | 1473.82 | 1473.82 | -0.00 | 0 | R.RLEKETGIPVVSF.D | (Ions score 80) |
| 362 - 380 | 2076.02 | 2075.01 | 2075.02 | -0.00 | 0 | R.RLEKETGIPVVSFDGDQAD.P | (Ions score 86) |
| 362 - 382 | 2329.20 | 2328.19 | 2328.17 | 0.02 | 0 | R.RLEKETGIPVVSFDGDQADPR.N | (Ions score 76) |
| 362 - 382 | 2329.26 | 2328.25 | 2328.17 | 0.08 | 0 | R.RLEKETGIPVVSFDGDQADPR.N | (Ions score 93) |
| 362 - 384 | 2590.31 | 2589.30 | 2589.28 | 0.02 | 0 | R.RLEKETGIPVVSFDGDQADPRNF.S | (Ions score 54) |
| 363 - 382 | 2173.09 | 2172.08 | 2172.07 | 0.01 | 0 | R.LEKETGIPVVSFDGDQADPR.N | (Ions score 39) |
| 363 - 382 | 2173.10 | 2172.09 | 2172.07 | 0.02 | 0 | R.LEKETGIPVVSFDGDQADPR.N | (Ions score 163) |
| 366 - 382 | 1802.87 | 1801.87 | 1801.85 | 0.02 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 142) |
| 366 - 382 | 1802.91 | 1801.91 | 1801.85 | 0.06 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 141) |
| 366 - 384 | 2063.99 | 2062.98 | 2062.96 | 0.02 | 0 | K.ETGIPVVSFDGDQADPRNF.S | (Ions score 38) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 367 - 382 | 1673.87 | 1672.86 | 1672.81 | 0.06 | 0 | **E.TGIPVVSFDGDQADPR.N** (Ions score 43) |
| 383 - 392 | 1230.53 | 1229.52 | 1229.53 | -0.01 | 0 | **R.NFSEAQYDTR.I** (Ions score 82) |
| 383 - 403 | 2413.21 | 2412.20 | 2412.17 | 0.03 | 0 | **R.NFSEAQYDTRIQGLNEVMVAK.K** (Ions score 57) |
| 383 - 404 | 2541.32 | 2540.32 | 2540.27 | 0.05 | 0 | **R.NFSEAQYDTRIQGLNEVMVAKK.E** (Ions score 41) |
| 383 - 407 | 2870.45 | 2869.44 | 2869.39 | 0.05 | 0 | **R.NFSEAQYDTRIQGLNEVMVAKKEAE.-** (Ions score 93) |
| 384 - 392 | 1116.48 | 1115.48 | 1115.49 | -0.01 | 0 | **N.FSEAQYDTR.I** (Ions score 18) |
| 390 - 400 | 1275.62 | 1274.61 | 1274.63 | -0.02 | 0 | **Y.DTRIQGLNEVM.V** (Ions score 35) |
| 393 - 400 | 903.43 | 902.42 | 902.45 | -0.03 | 0 | **R.IQGLNEVM.V** (Ions score 55) |
| 393 - 403 | 1201.64 | 1200.63 | 1200.65 | -0.02 | 0 | **R.IQGLNEVMVAK.K** (Ions score 79) |
| 393 - 407 | 1658.90 | 1657.89 | 1657.87 | 0.02 | 0 | **R.IQGLNEVMVAKKEAE.-** (Ions score 123) |

### Protein View of the small subunit FldC of dehydratase from Trp-culture

Match to: **R-phenyllactate-small-su** Score: **7270**
**dehydratase small subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **43120**; Calculated pI value: **5.17**
NCBI BLAST search of R-phenyllactate-small-su against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **78%**

Matched peptides shown in **Bold**

```
   1 MSNSDKFFND FKDIVENPKK YIMKHMEQTG QKAIGCMPLY TPEELVLAAG
  51 MFPVGVWGSN TELSKAKTYF PAFICSILQT TLENALNGEY DMLSGMMITN
 101 YCDSLKCMGQ NFKLTVENIE FIPVTVPQNR KMEAGKEFLK SQYKMNIEQL
 151 EKISGNKITD ESLEKAIEIY DEHRKVMNDF SMLASKYPGI ITPTKRNYVM
 201 KSAYYMDKKE HTEKVRQLMD EIKAIEPKPF EGKRVITTGI IADSEDLLKI
 251 LEENNIAIVG DDIAHESRQY RTLTPEANTP MDRLAEQFAN RECSTLYDPE
 301 KKRGQYIVEM AKERKADGII FFMTKFCDPE EYDYPQMKKD FEEAGIPHVL
 351 IETDMQMKNY EQARTAIQAF SETL
```

### Protein View of the large subunit FldA of dehydratase from Trp-culture

Match to: **E-cinnamoyl-CoA:R-phenyllactate** Score: **5179**
**CoA transferase large subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **46357**; Calculated pI value: **4.98**
NCBI BLAST search of E-cinnamoyl-CoA:R-phenyllactate against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **80%**

Matched peptides shown in **Bold**

```
   1 MENNTNMFSG VKVIELANFI AAPAAGRFFA DGGAEVIKIE SPAGDPLRYT
  51 APSEGRPLSQ EENTTYDLEN ANKKAIVLNL KSEKGKKILH EMLAEADILL
 101 TNWRTKALVK QGLDYETLKE KYPKLVFAQI TGYGEKGPDK DLPGFDYTAF
 151 FARGGVSGTL YEKGTVPPNV VPGLGDHQAG MFLAAGMAGA LYKAKTTGQG
 201 DKVTVSLMHS AMYGLGIMIQ AAQYKDHGLV YPINRNETPN PFIVSYKSKD
 251 DYFVQVCMPP YDVFYDRFMT ALGREDLVGD ERYNKIENLK DGRAKEVYSI
 301 IEQQMVTKTK DEWDNIFRDA DIPFAIAQTW EDLLEDEQAW ANDYLYKMKY
 351 PTGNERALVR LPVFFKEAGL PEYNQSPQIA ENTVEVLKEM GYTEQEIEEL
 401 EKDKDIMVRK EK
```

## *2. The dehydratase from the phenylalaine-culture*

**Protein View of the medium subunit FldB of dehydratase from Phe-culture**

Match to: **R-phenyllactate-medium-su** Score: **7324**
**dehydratase medium subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **46208**; Calculated pI value: **5.75**
NCBI BLAST search of R-phenyllactate-medium-su against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **77%**

Matched peptides shown in **Bold**

```
     1 MSDRNKEVKE KKAKHYLREI TAKHYKEALE AKERGEKVGW CASNFPQEIA
    51 TTLGVKVVYP ENHAAAVAAR GNGQNMCEHA EAMGFSNDVC GYARVNLAVM
   101 DIGHSEDQPI PMPDFVLCCN NICNQMIKWY EHIAKTLDIP MILIDIPYNT
   151 ENTVSQDRIK YIRAQFDDAI KQLEEITGKK WDENKFEEVM KISQESAKQW
   201 LRAASYAKYK PSPFSGFDLF NHMAVAVCAR GTQEAADAFK MLADEYEENV
   251 KTGKSTYRGE EKQRILFEGI ACWPYLRHKL TKLSEYGMNV TATVYAEAFG
   301 VIYENMDELM AAYNKVPNSI SFENALKMRL NAVTSTNTEG AVIHINRSCK
   351 LWSGFLYELA RRLEKETGIP VVSFDGDQAD PRNFSEAQYD TRIQGLNEVM
   401 VAKKEAE
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| Start - End | Observed | Mr(expt) | Mr(calc) | Delta | Miss | Sequence |
|---|---|---|---|---|---|---|
| 2 - 11 | 1232.64 | 1231.64 | 1231.65 | -0.01 | 0 | M.SDRNKEVKEK.K (Ions score 37) |
| 2 - 11 | 1232.65 | 1231.64 | 1231.65 | -0.01 | 0 | M.SDRNKEVKEK.K (Ions score 48) |
| 2 - 12 | 1360.74 | 1359.73 | 1359.75 | -0.02 | 0 | M.SDRNKEVKEKK.A (Ions score 74) |
| 2 - 14 | 1559.87 | 1558.86 | 1558.88 | -0.02 | 0 | M.SDRNKEVKEKKAK.H (Ions score 41) |
| 2 - 16 | 1860.01 | 1859.00 | 1859.00 | -0.00 | 0 | M.SDRNKEVKEKKAKHY.L (Ions score 19) |
| 13 - 23 | 1329.75 | 1328.74 | 1328.76 | -0.02 | 0 | K.AKHYLREITAK.H (Ions score 28) |
| 13 - 23 | 1329.78 | 1328.77 | 1328.76 | 0.02 | 0 | K.AKHYLREITAK.H (Ions score 19) |
| 13 - 23 | 1329.78 | 1328.77 | 1328.76 | 0.02 | 0 | K.AKHYLREITAK.H (Ions score 19) |
| 15 - 23 | 1130.61 | 1129.60 | 1129.62 | -0.02 | 0 | K.HYLREITAK.H (Ions score 39) |
| 15 - 23 | 1130.61 | 1129.60 | 1129.62 | -0.02 | 0 | K.HYLREITAK.H (Ions score 39) |
| 15 - 23 | 1130.63 | 1129.62 | 1129.62 | -0.00 | 0 | K.HYLREITAK.H (Ions score 45) |
| 15 - 23 | 1130.63 | 1129.62 | 1129.62 | 0.00 | 0 | K.HYLREITAK.H (Ions score 42) |
| 19 - 34 | 1915.99 | 1914.98 | 1915.02 | -0.03 | 0 | R.EITAKHYKEALEAKER.G (Ions score 41) |
| 19 - 34 | 1915.99 | 1914.98 | 1915.02 | -0.03 | 0 | R.EITAKHYKEALEAKER.G (Ions score 41) |
| 19 - 34 | 1916.04 | 1915.04 | 1915.02 | 0.02 | 0 | R.EITAKHYKEALEAKER.G (Ions score 91) |
| 19 - 34 | 1916.04 | 1915.04 | 1915.02 | 0.02 | 0 | R.EITAKHYKEALEAKER.G (Ions score 91) |
| 19 - 37 | 2230.17 | 2229.16 | 2229.18 | -0.02 | 0 | R.EITAKHYKEALEAKERGEK.V (Ions score 65) |
| 19 - 37 | 2230.17 | 2229.16 | 2229.18 | -0.02 | 0 | R.EITAKHYKEALEAKERGEK.V (Ions score 65) |
| 19 - 37 | 2230.21 | 2229.20 | 2229.18 | 0.02 | 0 | R.EITAKHYKEALEAKERGEK.V (Ions score 116) |
| 23 - 34 | 1501.81 | 1500.80 | 1500.80 | -0.00 | 0 | A.KHYKEALEAKER.G (Ions score 83) |
| 24 - 32 | 1088.57 | 1087.56 | 1087.57 | -0.01 | 0 | K.HYKEALEAK.E (Ions score 84) |
| 24 - 32 | 1088.57 | 1087.56 | 1087.57 | -0.01 | 0 | K.HYKEALEAK.E (Ions score 78) |
| 24 - 32 | 1088.57 | 1087.56 | 1087.57 | -0.01 | 0 | K.HYKEALEAK.E (Ions score 78) |
| 24 - 34 | 1373.71 | 1372.70 | 1372.71 | -0.01 | 0 | K.HYKEALEAKER.G (Ions score 91) |
| 24 - 34 | 1373.73 | 1372.73 | 1372.71 | 0.02 | 0 | K.HYKEALEAKER.G (Ions score 94) |
| 24 - 37 | 1687.87 | 1686.87 | 1686.87 | -0.00 | 0 | K.HYKEALEAKERGEK.V (Ions score 118) |
| 24 - 37 | 1687.88 | 1686.88 | 1686.87 | 0.01 | 0 | K.HYKEALEAKERGEK.V (Ions score 115) |
| 24 - 40 | 2030.05 | 2029.05 | 2029.04 | 0.01 | 0 | K.HYKEALEAKERGEKVGW.C (Ions score 106) |
| 24 - 42 | 2309.12 | 2308.12 | 2308.14 | -0.03 | 0 | K.HYKEALEAKERGEKVGWCA.S S-pyridylethyl (C) (Ions score 12) |
| 24 - 56 | 3794.93 | 3793.92 | 3793.92 | -0.01 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 28) |
| 24 - 56 | 3794.93 | 3793.92 | 3793.92 | -0.01 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 28) |
| 24 - 56 | 3795.05 | 3794.04 | 3793.92 | 0.12 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 38) |
| 24 - 56 | 3795.05 | 3794.04 | 3793.92 | 0.12 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 38) |
| 26 - 34 | 1073.59 | 1072.58 | 1072.59 | -0.00 | 0 | Y.KEALEAKER.G (Ions score 27) |
| 26 - 37 | 1387.75 | 1386.74 | 1386.75 | -0.01 | 0 | Y.KEALEAKERGEK.V (Ions score 52) |
| 26 - 40 | 1729.93 | 1728.92 | 1728.92 | 0.00 | 0 | Y.KEALEAKERGEKVGW.C (Ions score 62) |
| 27 - 34 | 945.50 | 944.49 | 944.49 | -0.00 | 0 | K.EALEAKER.G (Ions score 42) |
| 27 - 34 | 945.50 | 944.49 | 944.49 | -0.00 | 0 | K.EALEAKER.G (Ions score 56) |
| 27 - 34 | 945.50 | 944.49 | 944.49 | -0.00 | 0 | K.EALEAKER.G (Ions score 56) |
| 27 - 37 | 1259.67 | 1258.66 | 1258.65 | 0.01 | 0 | K.EALEAKERGEK.V (Ions score 25) |
| 27 - 40 | 1601.84 | 1600.83 | 1600.82 | 0.01 | 0 | K.EALEAKERGEKVGW.C (Ions score 37) |
| 33 - 56 | 2725.38 | 2724.37 | 2724.37 | 0.00 | 0 | K.ERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 23) |
| 35 - 56 | 2440.22 | 2439.21 | 2439.23 | -0.02 | 0 | R.GEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 111) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 35 - 56 | 2440.22 | 2439.21 | 2439.23 | -0.02 | 0 | R.GEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 111) |
| 35 - 70 | 3888.96 | 3887.95 | 3887.98 | -0.03 | 0 | R.GEKVGWCASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 64) |
| 35 - 70 | 3888.96 | 3887.95 | 3887.98 | -0.03 | 0 | R.GEKVGWCASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 64) |
| 38 - 56 | 2126.05 | 2125.05 | 2125.07 | -0.02 | 0 | K.VGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 151) |
| 38 - 56 | 2126.05 | 2125.05 | 2125.07 | -0.02 | 0 | K.VGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 151) |
| 38 - 70 | 3574.85 | 3573.85 | 3573.82 | 0.03 | 0 | K.VGWCASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 162) |
| 38 - 70 | 3574.85 | 3573.85 | 3573.82 | 0.03 | 0 | K.VGWCASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 162) |
| 41 - 53 | 1499.73 | 1498.72 | 1498.71 | 0.01 | 0 | W.CASNFPQEIATTL.G S-pyridylethyl (C) (Ions score 43) |
| 42 - 56 | 1575.83 | 1574.83 | 1574.83 | -0.00 | 0 | C.ASNFPQEIATTLGVK.V (Ions score 42) |
| 43 - 56 | 1504.82 | 1503.81 | 1503.79 | 0.02 | 0 | A.SNFPQEIATTLGVK.V (Ions score 28) |
| 46 - 56 | 1156.65 | 1155.64 | 1155.65 | -0.01 | 0 | F.PQEIATTLGVK.V (Ions score 88) |
| 46 - 56 | 1156.65 | 1155.64 | 1155.65 | -0.01 | 0 | F.PQEIATTLGVK.V (Ions score 88) |
| 51 - 70 | 2067.10 | 2066.09 | 2066.13 | -0.03 | 0 | A.TTLGVKVVYPENHAAAVAAR.G (Ions score 56) |
| 52 - 70 | 1966.09 | 1965.08 | 1965.08 | 0.00 | 0 | T.TLGVKVVYPENHAAAVAAR.G (Ions score 51) |
| 54 - 70 | 1751.94 | 1750.93 | 1750.95 | -0.02 | 0 | L.GVKVVYPENHAAAVAAR.G (Ions score 104) |
| 54 - 70 | 1751.96 | 1750.95 | 1750.95 | 0.00 | 0 | L.GVKVVYPENHAAAVAAR.G (Ions score 146) |
| 54 - 70 | 1751.98 | 1750.97 | 1750.95 | 0.03 | 0 | L.GVKVVYPENHAAAVAAR.G (Ions score 154) |
| 54 - 70 | 1751.98 | 1750.97 | 1750.95 | 0.03 | 0 | L.GVKVVYPENHAAAVAAR.G (Ions score 154) |
| 54 - 83 | 3229.53 | 3228.52 | 3228.50 | 0.02 | 0 | L.GVKVVYPENHAAAVAARGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 13) |
| 56 - 70 | 1595.87 | 1594.86 | 1594.86 | 0.00 | 0 | V.KVVYPENHAAAVAAR.G (Ions score 79) |
| 57 - 68 | 1240.63 | 1239.63 | 1239.62 | 0.00 | 0 | K.VVYPENHAAAVA.A (Ions score 49) |
| 57 - 70 | 1467.75 | 1466.74 | 1466.76 | -0.02 | 0 | K.VVYPENHAAAVAAR.G (Ions score 92) |
| 57 - 70 | 1467.81 | 1466.80 | 1466.76 | 0.04 | 0 | K.VVYPENHAAAVAAR.G (Ions score 136) |
| 57 - 70 | 1467.81 | 1466.80 | 1466.76 | 0.04 | 0 | K.VVYPENHAAAVAAR.G (Ions score 136) |
| 57 - 83 | 2945.34 | 2944.33 | 2944.32 | 0.01 | 0 | K.VVYPENHAAAVAARGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 64) |
| 57 - 83 | 2946.34 | 2945.33 | 2945.30 | 0.03 | 0 | K.VVYPENHAAAVAARGNGQNMCEHAEAM.G Deamidation (NQ); S-pyridylethyl (C) (Ions score 18) |
| 57 - 86 | 3149.42 | 3148.41 | 3148.34 | 0.07 | 0 | K.VVYPENHAAAVAARGNGQNMCEHAEAMGFS.N 2 Deamidation (NQ); Oxidation (M) (Ions score 19) |
| 58 - 70 | 1368.69 | 1367.69 | 1367.69 | -0.01 | 0 | V.VYPENHAAAVAAR.G (Ions score 47) |
| 58 - 70 | 1368.70 | 1367.69 | 1367.69 | -0.01 | 0 | V.VYPENHAAAVAAR.G (Ions score 52) |
| 62 - 70 | 880.47 | 879.46 | 879.47 | -0.00 | 0 | E.NHAAAVAAR.G (Ions score 33) |
| 69 - 83 | 1723.72 | 1722.72 | 1722.70 | 0.01 | 0 | A.ARGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 52) |
| 69 - 85 | 1928.77 | 1927.76 | 1927.78 | -0.01 | 0 | A.ARGNGQNMCEHAEAMGF.S Deamidation (NQ); S-pyridylethyl (C) (Ions score 26) |
| 69 - 94 | 2998.23 | 2997.22 | 2997.25 | -0.03 | 0 | A.ARGNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 23) |
| 69 - 94 | 2998.23 | 2997.22 | 2997.25 | -0.03 | 0 | A.ARGNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 23) |
| 69 - 94 | 2998.28 | 2997.27 | 2997.25 | 0.02 | 0 | A.ARGNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 16) |
| 69 - 94 | 2998.28 | 2997.27 | 2997.25 | 0.02 | 0 | A.ARGNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 16) |
| 70 - 83 | 1652.67 | 1651.66 | 1651.67 | -0.00 | 0 | A.RGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 27) |
| 70 - 83 | 1652.67 | 1651.66 | 1651.67 | -0.00 | 0 | A.RGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 27) |
| 70 - 83 | 1652.69 | 1651.68 | 1651.67 | 0.01 | 0 | A.RGNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 40) |
| 70 - 85 | 1856.73 | 1855.73 | 1855.76 | -0.03 | 0 | A.RGNGQNMCEHAEAMGF.S S-pyridylethyl (C) (Ions score 48) |
| 71 - 80 | 1165.44 | 1164.43 | 1164.44 | -0.01 | 0 | R.GNGQNMCEHA.E S-pyridylethyl (C) (Ions score 41) |
| 71 - 80 | 1165.45 | 1164.45 | 1164.44 | 0.00 | 0 | R.GNGQNMCEHA.E S-pyridylethyl (C) (Ions score 56) |
| 71 - 81 | 1294.46 | 1293.45 | 1293.49 | -0.03 | 0 | R.GNGQNMCEHAE.A S-pyridylethyl (C) (Ions score 49) |
| 71 - 83 | 1496.57 | 1495.56 | 1495.56 | 0.00 | 0 | R.GNGQNMCEHAEAM.G S-pyridylethyl (C) (Ions score 68) |
| 71 - 83 | 1497.51 | 1496.50 | 1496.55 | -0.04 | 0 | R.GNGQNMCEHAEAM.G Deamidation (NQ); S-pyridylethyl (C) (Ions score 8) |
| 71 - 83 | 1513.55 | 1512.54 | 1512.54 | -0.00 | 0 | R.GNGQNMCEHAEAM.G Deamidation (NQ); Oxidation (M); S-pyridylethyl (C) (Ions score 26) |
| 71 - 85 | 1700.62 | 1699.61 | 1699.65 | -0.04 | 0 | R.GNGQNMCEHAEAMGF.S S-pyridylethyl (C) (Ions score 51) |
| 71 - 85 | 1700.62 | 1699.61 | 1699.65 | -0.04 | 0 | R.GNGQNMCEHAEAMGF.S S-pyridylethyl (C) (Ions score 51) |
| 71 - 85 | 1700.64 | 1699.63 | 1699.65 | -0.02 | 0 | R.GNGQNMCEHAEAMGF.S S-pyridylethyl (C) (Ions score 81) |
| 71 - 85 | 1701.62 | 1700.61 | 1700.64 | -0.03 | 0 | R.GNGQNMCEHAEAMGF.S Deamidation (NQ); S-pyridylethyl (C) (Ions score 43) |
| 71 - 85 | 1716.64 | 1715.63 | 1715.65 | -0.02 | 0 | R.GNGQNMCEHAEAMGF.S Oxidation (M); S-pyridylethyl (C) (Ions score 59) |
| 71 - 92 | 2349.84 | 2348.84 | 2348.86 | -0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGY.A Oxidation (M) (Ions score 6) |
| 71 - 94 | 2666.01 | 2665.01 | 2665.06 | -0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V S-pyridylethyl (C) (Ions score 56) |
| 71 - 94 | 2666.99 | 2665.98 | 2666.04 | -0.06 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V Deamidation (NQ); S-pyridylethyl (C) (Ions score 32) |
| 71 - 94 | 2666.99 | 2665.98 | 2666.04 | -0.06 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V Deamidation (NQ); S-pyridylethyl (C) (Ions score 32) |
| 71 - 94 | 2668.04 | 2667.03 | 2667.02 | 0.00 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 Deamidation (NQ); S-pyridylethyl (C) (Ions score 24) |
| 71 - 94 | 2668.04 | 2667.03 | 2667.02 | 0.00 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 Deamidation (NQ); S-pyridylethyl (C) (Ions score 24) |
| 71 - 94 | 2771.11 | 2770.10 | 2770.11 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 97) |
| 71 - 94 | 2771.13 | 2770.12 | 2770.11 | 0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 83) |
| 71 - 94 | 2771.15 | 2770.14 | 2770.11 | 0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 153) |
| 71 - 94 | 2771.15 | 2770.14 | 2770.11 | 0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 153) |
| 71 - 94 | 2771.19 | 2770.18 | 2770.11 | 0.07 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V 2 S-pyridylethyl (C) (Ions score 148) |
| 71 - 94 | 2772.10 | 2771.09 | 2771.10 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V Deamidation (NQ); 2 S-pyridylethyl (C) (Ions score 34) |
| 71 - 94 | 2772.10 | 2771.09 | 2771.10 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V Deamidation (NQ); 2 S-pyridylethyl (C) (Ions score 149) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 71 - 94 | 2772.16 | 2771.15 | 2771.10 | 0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); 2 S-pyridylethyl (C) (Ions score 136) |
| 71 - 94 | 2787.10 | 2786.09 | 2786.11 | -0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 114) |
| 71 - 94 | 2787.10 | 2786.09 | 2786.11 | -0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 114) |
| 71 - 94 | 2787.15 | 2786.14 | 2786.11 | 0.04 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 92) |
| 71 - 94 | 2788.09 | 2787.08 | 2787.09 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); Oxidation (M); 2 S-pyridylethyl (C) (Ions score 98) |
| 71 - 94 | 2788.17 | 2787.16 | 2787.09 | 0.07 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); Oxidation (M); 2 S-pyridylethyl (C) (Ions score 101) |
| 71 - 94 | 2788.17 | 2787.16 | 2787.09 | 0.07 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); Oxidation (M); 2 S-pyridylethyl (C) (Ions score 101) |
| 71 - 94 | 2803.08 | 2802.07 | 2802.10 | -0.03 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 Oxidation (M); 2 S-pyridylethyl (C) (Ions score 46) |
| 71 - 94 | 2803.08 | 2802.07 | 2802.10 | -0.03 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 Oxidation (M); 2 S-pyridylethyl (C) (Ions score 46) |
| 73 - 94 | 2600.02 | 2599.01 | 2599.05 | -0.04 | 0 | N.GQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 40) |
| 73 - 94 | 2600.02 | 2599.01 | 2599.05 | -0.04 | 0 | N.GQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 40) |
| 74 - 94 | 2541.99 | 2540.98 | 2541.00 | -0.01 | 0 | G.QNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); Pyro-glu (N-term Q); 2 S-pyridylethyl (C) (Ions score 12) |
| 74 - 94 | 2541.99 | 2540.98 | 2541.00 | -0.01 | 0 | G.QNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); Pyro-glu (N-term Q); 2 S-pyridylethyl (C) (Ions score 12) |
| 74 - 94 | 2558.97 | 2557.96 | 2557.98 | -0.01 | 0 | G.QNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); 2 Oxidation (M); Pyro-glu (N-term Q); 2 S-pyridylethyl (C) (Ions score 13) |
| 82 - 94 | 1495.61 | 1494.60 | 1494.64 | -0.03 | 0 | E.AMGFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 32) |
| 84 - 92 | 1066.40 | 1065.39 | 1065.42 | -0.03 | 0 | M.GFSNDVCGY.A | S-pyridylethyl (C) (Ions score 38) |
| 84 - 94 | 1293.56 | 1292.55 | 1292.56 | -0.01 | 0 | M.GFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 80) |
| 84 - 94 | 1293.57 | 1292.57 | 1292.56 | 0.01 | 0 | M.GFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 81) |
| 86 - 94 | 1089.49 | 1088.48 | 1088.47 | 0.01 | 0 | F.SNDVCGYAR.V | S-pyridylethyl (C) (Ions score 25) |
| 88 - 94 | 888.40 | 887.39 | 887.40 | -0.01 | 0 | N.DVCGYAR.V | S-pyridylethyl (C) (Ions score 34) |
| 136 - 158 | 2661.28 | 2660.28 | 2660.34 | -0.06 | 0 | K.TLDIPMILIDIPYNTENTVSQDR.I | (Ions score 158) |
| 136 - 160 | 2902.45 | 2901.45 | 2901.52 | -0.07 | 0 | K.TLDIPMILIDIPYNTENTVSQDRIK.Y | (Ions score 80) |
| 136 - 160 | 2902.45 | 2901.45 | 2901.52 | -0.07 | 0 | K.TLDIPMILIDIPYNTENTVSQDRIK.Y | (Ions score 80) |
| 149 - 158 | 1163.51 | 1162.50 | 1162.52 | -0.02 | 0 | Y.NTENTVSQDR.I | (Ions score 33) |
| 161 - 179 | 2238.20 | 2237.19 | 2237.17 | 0.02 | 0 | K.YIRAQFDDAIKQLEEITGK.K | (Ions score 50) |
| 161 - 180 | 2366.27 | 2365.26 | 2365.26 | -0.00 | 0 | K.YIRAQFDDAIKQLEEITGKK.W | (Ions score 41) |
| 161 - 181 | 2552.38 | 2551.37 | 2551.34 | 0.03 | 0 | K.YIRAQFDDAIKQLEEITGKKW.D | (Ions score 40) |
| 164 - 171 | 907.43 | 906.42 | 906.44 | -0.03 | 0 | R.AQFDDAIK.Q | (Ions score 34) |
| 164 - 171 | 907.43 | 906.42 | 906.44 | -0.03 | 0 | R.AQFDDAIK.Q | (Ions score 34) |
| 164 - 171 | 907.45 | 906.44 | 906.44 | -0.01 | 0 | R.AQFDDAIK.Q | (Ions score 77) |
| 164 - 171 | 907.45 | 906.44 | 906.44 | -0.01 | 0 | R.AQFDDAIK.Q | (Ions score 77) |
| 164 - 179 | 1805.90 | 1804.89 | 1804.92 | -0.03 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 118) |
| 164 - 179 | 1805.93 | 1804.92 | 1804.92 | 0.00 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 74) |
| 164 - 179 | 1805.93 | 1804.92 | 1804.92 | 0.00 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 74) |
| 164 - 180 | 1933.96 | 1932.95 | 1933.02 | -0.06 | 0 | R.AQFDDAIKQLEEITGKK.W | (Ions score 48) |
| 164 - 180 | 1933.96 | 1932.95 | 1933.02 | -0.06 | 0 | R.AQFDDAIKQLEEITGKK.W | (Ions score 48) |
| 164 - 181 | 2120.08 | 2119.08 | 2119.09 | -0.02 | 0 | R.AQFDDAIKQLEEITGKKW.D | (Ions score 61) |
| 164 - 191 | 3369.65 | 3368.64 | 3368.66 | -0.02 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 78) |
| 164 - 191 | 3369.65 | 3368.64 | 3368.66 | -0.02 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 78) |
| 164 - 191 | 3369.66 | 3368.65 | 3368.66 | -0.01 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 95) |
| 164 - 191 | 3385.71 | 3384.70 | 3384.65 | 0.05 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | Oxidation (M) (Ions score 27) |
| 180 - 191 | 1582.74 | 1581.73 | 1581.75 | -0.02 | 0 | K.KWDENKFEEVMK.I | (Ions score 64) |
| 180 - 202 | 2909.44 | 2908.43 | 2908.45 | -0.02 | 0 | K.KWDENKFEEVMKISQESAKQWLR.A | (Ions score 191) |
| 180 - 202 | 2909.44 | 2908.43 | 2908.45 | -0.02 | 0 | K.KWDENKFEEVMKISQESAKQWLR.A | (Ions score 191) |
| 181 - 191 | 1454.63 | 1453.63 | 1453.65 | -0.03 | 0 | K.WDENKFEEVMK.I | (Ions score 24) |
| 181 - 191 | 1454.66 | 1453.65 | 1453.65 | -0.00 | 0 | K.WDENKFEEVMK.I | (Ions score 50) |
| 181 - 191 | 1454.66 | 1453.65 | 1453.65 | -0.00 | 0 | K.WDENKFEEVMK.I | (Ions score 50) |
| 181 - 202 | 2781.29 | 2780.29 | 2780.36 | -0.07 | 0 | K.WDENKFEEVMKISQESAKQWLR.A | (Ions score 143) |
| 192 - 200 | 1076.52 | 1075.51 | 1075.53 | -0.02 | 0 | K.ISQESAKQW.L | (Ions score 37) |
| 192 - 202 | 1345.71 | 1344.71 | 1344.71 | -0.01 | 0 | K.ISQESAKQWLR.A | (Ions score 101) |
| 209 - 224 | 1857.83 | 1856.83 | 1856.86 | -0.03 | 0 | K.YKPSPFSGFDLFNHMA.V | (Ions score 66) |
| 209 - 224 | 1857.83 | 1856.83 | 1856.86 | -0.03 | 0 | K.YKPSPFSGFDLFNHMA.V | (Ions score 66) |
| 209 - 230 | 2562.22 | 2561.21 | 2561.23 | -0.02 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G | S-pyridylethyl (C) (Ions score 65) |
| 209 - 230 | 2562.22 | 2561.21 | 2561.23 | -0.02 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G | S-pyridylethyl (C) (Ions score 65) |
| 215 - 230 | 1842.86 | 1841.86 | 1841.87 | -0.01 | 0 | F.SGFDLFNHMAVAVCAR.G | S-pyridylethyl (C) (Ions score 73) |
| 215 - 230 | 1842.86 | 1841.86 | 1841.87 | -0.01 | 0 | F.SGFDLFNHMAVAVCAR.G | S-pyridylethyl (C) (Ions score 73) |
| 231 - 240 | 1037.48 | 1036.47 | 1036.48 | -0.01 | 0 | R.GTQEAADAFK.M | (Ions score 69) |
| 231 - 240 | 1037.49 | 1036.48 | 1036.48 | -0.00 | 0 | R.GTQEAADAFK.M | (Ions score 71) |
| 231 - 240 | 1037.49 | 1036.48 | 1036.48 | -0.00 | 0 | R.GTQEAADAFK.M | (Ions score 71) |
| 231 - 254 | 2645.29 | 2644.28 | 2644.23 | 0.05 | 0 | R.GTQEAADAFKMLADEYEENVKTGK.S | (Ions score 151) |
| 241 - 251 | 1340.61 | 1339.60 | 1339.60 | 0.00 | 0 | K.MLADEYEENVK.T | (Ions score 75) |
| 241 - 251 | 1340.61 | 1339.60 | 1339.60 | 0.00 | 0 | K.MLADEYEENVK.T | (Ions score 75) |
| 241 - 251 | 1340.63 | 1339.63 | 1339.60 | 0.03 | 0 | K.MLADEYEENVK.T | (Ions score 28) |
| 241 - 254 | 1626.75 | 1625.74 | 1625.76 | -0.02 | 0 | K.MLADEYEENVKTGK.S | (Ions score 78) |
| 241 - 254 | 1626.75 | 1625.75 | 1625.76 | -0.01 | 0 | K.MLADEYEENVKTGK.S | (Ions score 78) |
| 241 - 264 | 2861.34 | 2860.33 | 2860.37 | -0.04 | 0 | K.MLADEYEENVKTGKSTYRGEEKQR.I | (Ions score 92) |
| 241 - 264 | 2861.34 | 2860.33 | 2860.37 | -0.04 | 0 | K.MLADEYEENVKTGKSTYRGEEKQR.I | (Ions score 92) |
| 254 - 264 | 1381.73 | 1380.72 | 1380.71 | 0.01 | 0 | G.KSTYRGEEKQR.I | (Ions score 19) |
| 255 - 262 | 969.47 | 968.46 | 968.46 | 0.00 | 0 | K.STYRGEEK.Q | (Ions score 35) |
| 255 - 264 | 1253.62 | 1252.61 | 1252.62 | -0.01 | 0 | K.STYRGEEKQR.I | (Ions score 37) |
| 255 - 264 | 1253.62 | 1252.61 | 1252.62 | -0.00 | 0 | K.STYRGEEKQR.I | (Ions score 49) |
| 255 - 264 | 1253.62 | 1252.61 | 1252.62 | -0.00 | 0 | K.STYRGEEKQR.I | (Ions score 49) |
| 258 - 264 | 902.47 | 901.46 | 901.47 | -0.01 | 0 | Y.RGEEKQR.I | (Ions score 18) |
| 267 - 277 | 1459.71 | 1458.70 | 1458.71 | -0.01 | 0 | L.FEGIACWPYLR.H | S-pyridylethyl (C) (Ions score 31) |
| 267 - 277 | 1459.71 | 1458.70 | 1458.71 | -0.01 | 0 | L.FEGIACWPYLR.H | S-pyridylethyl (C) (Ions score 31) |
| 267 - 277 | 1459.74 | 1458.74 | 1458.71 | 0.02 | 0 | L.FEGIACWPYLR.H | S-pyridylethyl (C) (Ions score 43) |
| 267 - 277 | 1459.74 | 1458.74 | 1458.71 | 0.02 | 0 | L.FEGIACWPYLR.H | S-pyridylethyl (C) (Ions score 43) |
| 315 - 324 | 1136.59 | 1135.58 | 1135.54 | 0.04 | 0 | N.KVPNSISFEN.A | 2 Deamidation (NQ) (Ions score 26) |
| 316 - 327 | 1318.67 | 1317.66 | 1317.69 | -0.03 | 0 | K.VPNSISFENALK.M | (Ions score 50) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 316 - 327 | 1318.67 | 1317.66 | 1317.69 | -0.03 | 0 | K.VPNSISFENALK.M | (Ions score 50) |
| 316 - 329 | 1605.81 | 1604.80 | 1604.83 | -0.03 | 0 | K.VPNSISFENALKMR.L | (Ions score 44) |
| 328 - 347 | 2197.14 | 2196.13 | 2196.14 | -0.01 | 0 | K.MRLNAVTSTNTEGAVIHINR.S | (Ions score 47) |
| 328 - 347 | 2197.16 | 2196.16 | 2196.14 | 0.01 | 0 | K.MRLNAVTSTNTEGAVIHINR.S | (Ions score 78) |
| 328 - 347 | 2197.16 | 2196.16 | 2196.14 | 0.01 | 0 | K.MRLNAVTSTNTEGAVIHINR.S | (Ions score 78) |
| 329 - 347 | 2066.08 | 2065.07 | 2065.10 | -0.03 | 0 | M.RLNAVTSTNTEGAVIHINR.S | (Ions score 50) |
| 330 - 347 | 1909.97 | 1908.96 | 1909.00 | -0.04 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 59) |
| 330 - 347 | 1909.98 | 1908.97 | 1909.00 | -0.03 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 169) |
| 330 - 347 | 1909.98 | 1908.97 | 1909.00 | -0.03 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 156) |
| 330 - 347 | 1910.01 | 1909.00 | 1909.00 | 0.00 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 160) |
| 330 - 347 | 1910.01 | 1909.00 | 1909.00 | 0.00 | 0 | R.LNAVTSTNTEGAVIHINR.S | (Ions score 160) |
| 331 - 347 | 1796.93 | 1795.92 | 1795.92 | 0.00 | 0 | L.NAVTSTNTEGAVIHINR.S | (Ions score 23) |
| 332 - 347 | 1682.86 | 1681.85 | 1681.87 | -0.02 | 0 | N.AVTSTNTEGAVIHINR.S | (Ions score 79) |
| 332 - 347 | 1682.88 | 1681.87 | 1681.87 | -0.00 | 0 | N.AVTSTNTEGAVIHINR.S | (Ions score 110) |
| 350 - 361 | 1482.79 | 1481.78 | 1481.80 | -0.02 | 0 | C.KLWSGFLYELAR.R | (Ions score 71) |
| 350 - 361 | 1482.79 | 1481.78 | 1481.80 | -0.02 | 0 | C.KLWSGFLYELAR.R | (Ions score 71) |
| 351 - 361 | 1354.70 | 1353.69 | 1353.71 | -0.01 | 0 | K.LWSGFLYELAR.R | (Ions score 104) |
| 351 - 361 | 1354.70 | 1353.69 | 1353.71 | -0.01 | 0 | K.LWSGFLYELAR.R | (Ions score 104) |
| 351 - 361 | 1354.71 | 1353.71 | 1353.71 | -0.00 | 0 | K.LWSGFLYELAR.R | (Ions score 87) |
| 352 - 361 | 1241.62 | 1240.61 | 1240.62 | -0.01 | 0 | L.WSGFLYELAR.R | (Ions score 85) |
| 352 - 361 | 1241.63 | 1240.62 | 1240.62 | -0.00 | 0 | L.WSGFLYELAR.R | (Ions score 27) |
| 361 - 382 | 2485.25 | 2484.24 | 2484.27 | -0.03 | 0 | A.RRLEKETGIPVVSFDGDQADPR.N | (Ions score 21) |
| 362 - 374 | 1474.83 | 1473.82 | 1473.82 | -0.00 | 0 | R.RLEKETGIPVVSF.D | (Ions score 65) |
| 362 - 380 | 2076.02 | 2075.01 | 2075.02 | -0.01 | 0 | R.RLEKETGIPVVSFDGDQAD.P | (Ions score 59) |
| 362 - 382 | 2329.19 | 2328.18 | 2328.17 | 0.01 | 0 | R.RLEKETGIPVVSFDGDQADPR.N | (Ions score 115) |
| 362 - 382 | 2329.19 | 2328.19 | 2328.17 | 0.02 | 0 | R.RLEKETGIPVVSFDGDQADPR.N | (Ions score 71) |
| 362 - 382 | 2329.24 | 2328.24 | 2328.17 | 0.07 | 0 | R.RLEKETGIPVVSFDGDQADPR.N | (Ions score 102) |
| 362 - 382 | 2329.24 | 2328.24 | 2328.17 | 0.07 | 0 | R.RLEKETGIPVVSFDGDQADPR.N | (Ions score 102) |
| 362 - 384 | 2590.33 | 2589.32 | 2589.28 | 0.04 | 0 | R.RLEKETGIPVVSFDGDQADPRNF.S | (Ions score 61) |
| 363 - 382 | 2173.03 | 2172.02 | 2172.07 | -0.05 | 0 | R.LEKETGIPVVSFDGDQADPR.N | (Ions score 108) |
| 363 - 382 | 2173.07 | 2172.07 | 2172.07 | -0.00 | 0 | R.LEKETGIPVVSFDGDQADPR.N | (Ions score 113) |
| 363 - 382 | 2173.11 | 2172.10 | 2172.07 | 0.03 | 0 | R.LEKETGIPVVSFDGDQADPR.N | (Ions score 159) |
| 364 - 382 | 2059.95 | 2058.95 | 2058.99 | -0.04 | 0 | L.EKETGIPVVSFDGDQADPR.N | (Ions score 85) |
| 364 - 382 | 2059.98 | 2058.97 | 2058.99 | -0.01 | 0 | L.EKETGIPVVSFDGDQADPR.N | (Ions score 122) |
| 365 - 382 | 1930.93 | 1929.93 | 1929.94 | -0.02 | 0 | E.KETGIPVVSFDGDQADPR.N | (Ions score 25) |
| 366 - 382 | 1802.81 | 1801.81 | 1801.85 | -0.04 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 73) |
| 366 - 382 | 1802.84 | 1801.83 | 1801.85 | -0.02 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 142) |
| 366 - 382 | 1802.84 | 1801.83 | 1801.85 | -0.02 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 142) |
| 366 - 382 | 1802.85 | 1801.84 | 1801.85 | -0.01 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 141) |
| 366 - 382 | 1802.88 | 1801.88 | 1801.85 | 0.03 | 0 | K.ETGIPVVSFDGDQADPR.N | (Ions score 138) |
| 366 - 384 | 2063.93 | 2062.92 | 2062.96 | -0.04 | 0 | K.ETGIPVVSFDGDQADPRNF.S | (Ions score 37) |
| 367 - 382 | 1673.78 | 1672.78 | 1672.81 | -0.03 | 0 | E.TGIPVVSFDGDQADPR.N | (Ions score 86) |
| 367 - 382 | 1673.78 | 1672.78 | 1672.81 | -0.03 | 0 | E.TGIPVVSFDGDQADPR.N | (Ions score 86) |
| 367 - 382 | 1673.81 | 1672.80 | 1672.81 | -0.00 | 0 | E.TGIPVVSFDGDQADPR.N | (Ions score 75) |
| 374 - 382 | 1020.43 | 1019.42 | 1019.43 | -0.01 | 0 | S.FDGDQADPR.N | (Ions score 32) |
| 374 - 382 | 1020.44 | 1019.43 | 1019.43 | -0.00 | 0 | S.FDGDQADPR.N | (Ions score 60) |
| 383 - 392 | 1230.54 | 1229.53 | 1229.53 | -0.00 | 0 | R.NFSEAQYDTR.I | (Ions score 77) |
| 383 - 392 | 1230.59 | 1229.58 | 1229.53 | 0.05 | 0 | R.NFSEAQYDTR.I | (Ions score 87) |
| 383 - 403 | 2413.18 | 2412.18 | 2412.17 | 0.00 | 0 | R.NFSEAQYDTRIQGLNEVMVAK.K | (Ions score 12) |
| 384 - 392 | 1116.49 | 1115.48 | 1115.49 | -0.01 | 0 | N.FSEAQYDTR.I | (Ions score 24) |
| 384 - 392 | 1116.49 | 1115.48 | 1115.49 | -0.01 | 0 | N.FSEAQYDTR.I | (Ions score 24) |
| 385 - 392 | 969.43 | 968.42 | 968.42 | 0.00 | 0 | F.SEAQYDTR.I | (Ions score 39) |
| 385 - 392 | 969.43 | 968.42 | 968.42 | 0.00 | 0 | F.SEAQYDTR.I | (Ions score 39) |
| 386 - 392 | 882.39 | 881.38 | 881.39 | -0.00 | 0 | S.EAQYDTR.I | (Ions score 27) |
| 386 - 392 | 882.39 | 881.39 | 881.39 | -0.00 | 0 | S.EAQYDTR.I | (Ions score 15) |
| 392 - 400 | 1059.57 | 1058.56 | 1058.55 | 0.00 | 0 | T.RIQGLNEVM.V | (Ions score 41) |
| 393 - 403 | 1201.63 | 1200.62 | 1200.65 | -0.03 | 0 | R.IQGLNEVMVAK.K | (Ions score 83) |
| 393 - 403 | 1201.63 | 1200.62 | 1200.65 | -0.03 | 0 | R.IQGLNEVMVAK.K | (Ions score 83) |
| 393 - 403 | 1201.63 | 1200.63 | 1200.65 | -0.03 | 0 | R.IQGLNEVMVAK.K | (Ions score 76) |
| 393 - 404 | 1329.73 | 1328.72 | 1328.75 | -0.03 | 0 | R.IQGLNEVMVAKK.E | (Ions score 48) |
| 393 - 404 | 1329.73 | 1328.72 | 1328.75 | -0.03 | 0 | R.IQGLNEVMVAKK.E | (Ions score 29) |
| 393 - 404 | 1329.75 | 1328.74 | 1328.75 | -0.01 | 0 | R.IQGLNEVMVAKK.E | (Ions score 75) |
| 393 - 404 | 1329.75 | 1328.74 | 1328.75 | -0.01 | 0 | R.IQGLNEVMVAKK.E | (Ions score 75) |
| 395 - 403 | 960.51 | 959.50 | 959.51 | -0.01 | 0 | Q.GLNEVMVAK.K | (Ions score 52) |

**Protein View of the small subunit FldC of dehydratase from Phe-culture**

Match to: **R-phenyllactate-small-su** Score: **12686**
**dehydratase small subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **43120**; Calculated pI value: **5.17**
NCBI BLAST search of R-phenyllactate-small-su against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **87%**

Matched peptides shown in **Bold**

```
    1 MSNSDKFFND FKDIVENPKK YIMKHMEQTG QKAIGCMPLY TPEELVLAAG
   51 MFPVGVWGSN TELSKAKTYF PAFICSILQT TLENALNGEY DMLSGMMITN
  101 YCDSLKCMGQ NFKLTVENIE FIPVTVPQNR KMEAGKEFLK SQYKMNIEQL
  151 EKISGNKITD ESLEKAIEIY DEHRKVMNDF SMLASKYPGI ITPTKRNYVM
  201 KSAYYMDKKE HTEKVRQLMD EIKAIEPKPF EGKRVITTGI IADSEDLLKI
  251 LEENNIAIVG DDIAHESRQY RTLTPEANTP MDRLAEQFAN RECSTLYDPE
  301 KKRGQYIVEM AKERKADGII FFMTKFCDPE EYDYPQMKKD FEEAGIPHVL
  351 IETDMQMKNY EQARTAIQAF SETL
```

**Protein View of the large subunit FldA of dehydratase from Phe-culture**

Match to: **E-cinnamoyl-CoA:R-phenyllactate** Score: **8669**
**CoA transferase large subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **46357**; Calculated pI value: **4.98**
NCBI BLAST search of E-cinnamoyl-CoA:R-phenyllactate against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **75%**

Matched peptides shown in **Bold**

```
    1 MENNTNMFSG VKVIELANFI AAPAAGRFFA DGGAEVIKIE SPAGDPLRYT
   51 APSEGRPLSQ EENTTYDLEN ANKKAIVLNL KSEKGKKILH EMLAEADILL
  101 TNWRTKALVK QGLDYETLKE KYPKLVFAQI TGYGEKGPDK DLPGFDYTAF
  151 FARGGVSGTL YEKGTVPPNV VPGLGDHQAG MFLAAGMAGA LYKAKTTGQG
  201 DKVTVSLMHS AMYGLGIMIQ AAQYKDHGLV YPINRNETPN PFIVSYKSKD
  251 DYFVQVCMPP YDVFYDRFMT ALGREDLVGD ERYNKIENLK DGRAKEVYSI
  301 IEQQMVTKTK DEWDNIFRDA DIPFAIAQTW EDLLEDEQAW ANDYLYKMKY
  351 PTGNERALVR LPVFFKEAGL PEYNQSPQIA ENTVEVLKEM GYTEQEIEEL
  401 EKDKDIMVRK EK
```

## *3. Recombinant dehydratase FldBC produced in E.coli*

**Protein View of the medium subunit FldB of the recombinant dehydratase**

Match to: **R-phenyllactate-medium-su** Score: **7386**
**dehydratase medium subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **46208**; Calculated pI value: **5.75**
NCBI BLAST search of R-phenyllactate-medium-su against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **74%**

Matched peptides shown in **Bold Red**

```
    1 MSDRNKEVKE KKAKHYLREI TAKHYKEALE AKERGEKVGW CASNFPQEIA
   51 TTLGVKVVYP ENHAAAVAAR GNGQNMCEHA EAMGFSNDVC GYARVNLAVM
  101 DIGHSEDQPI PMPDFVLCCN NICNQMIKWY EHIAKTLDIP MILIDIPYNT
  151 ENTVSQDRIK YIRAQFDDAI KQLEEITGKK WDENKFEEVM KISQESAKQW
  201 LRAASYAKYK PSPFSGFDLF NHMAVAVCAR GTQEAADAFK MLADEYEENV
  251 KTGKSTYRGE EKQRILFEGI ACWPYLRHKL TKLSEYGMNV TATVYAEAFG
  301 VIYENMDELM AAYNKVPNSI SFENALKMRL NAVTSTNTEG AVIHINRSCK
  351 LWSGFLYELA RRLEKETGIP VVSFDGDQAD PRNFSEAQYD TRIQGLNEVM
  401 VAKKEAE
```

| Start - End | Observed | Mr(expt) | Mr(calc) | Delta | Miss | Sequence |
|---|---|---|---|---|---|---|
| 12 - 18 | 915.54 | 914.54 | 914.54 | -0.01 | 0 | K.KAKHYLR.E (Ions score 22) |
| 12 - 18 | 915.55 | 914.54 | 914.54 | -0.00 | 0 | K.KAKHYLR.E (Ions score 22) |
| 13 - 23 | 1329.78 | 1328.77 | 1328.76 | 0.01 | 0 | K.AKHYLREITAK.H (Ions score 31) |
| 13 - 23 | 1329.78 | 1328.77 | 1328.76 | 0.02 | 0 | K.AKHYLREITAK.H (Ions score 23) |
| 15 - 23 | 1130.61 | 1129.60 | 1129.62 | -0.02 | 0 | K.HYLREITAK.H (Ions score 41) |
| 15 - 23 | 1130.63 | 1129.62 | 1129.62 | -0.00 | 0 | K.HYLREITAK.H (Ions score 43) |
| 15 - 23 | 1130.63 | 1129.62 | 1129.62 | -0.00 | 0 | K.HYLREITAK.H (Ions score 44) |
| 15 - 25 | 1430.77 | 1429.76 | 1429.75 | 0.02 | 0 | K.HYLREITAKHY.K (Ions score 23) |
| 15 - 26 | 1558.85 | 1557.84 | 1557.84 | 0.00 | 0 | K.HYLREITAKHYK.E (Ions score 31) |
| 15 - 26 | 1558.85 | 1557.84 | 1557.84 | 0.00 | 0 | K.HYLREITAKHYK.E (Ions score 62) |
| 15 - 32 | 2200.19 | 2199.19 | 2199.18 | 0.01 | 0 | K.HYLREITAKHYKEALEAK.E (Ions score 68) |
| 15 - 34 | 2485.39 | 2484.38 | 2484.32 | 0.06 | 0 | K.HYLREITAKHYKEALEAKER.G (Ions score 47) |
| 19 - 34 | 1916.00 | 1915.00 | 1915.02 | -0.02 | 0 | R.EITAKHYKEALEAKER.G (Ions score 92) |
| 24 - 32 | 1088.56 | 1087.55 | 1087.57 | -0.01 | 0 | K.HYKEALEAK.E (Ions score 71) |
| 24 - 32 | 1088.56 | 1087.55 | 1087.57 | -0.01 | 0 | K.HYKEALEAK.E (Ions score 78) |
| 24 - 32 | 1088.57 | 1087.56 | 1087.57 | -0.00 | 0 | K.HYKEALEAK.E (Ions score 73) |
| 24 - 34 | 1373.70 | 1372.70 | 1372.71 | -0.01 | 0 | K.HYKEALEAKER.G (Ions score 96) |
| 24 - 34 | 1373.73 | 1372.73 | 1372.71 | 0.02 | 0 | K.HYKEALEAKER.G (Ions score 94) |
| 24 - 34 | 1373.74 | 1372.73 | 1372.71 | 0.02 | 0 | K.HYKEALEAKER.G (Ions score 99) |
| 24 - 37 | 1687.87 | 1686.87 | 1686.87 | -0.00 | 0 | K.HYKEALEAKERGEK.V (Ions score 93) |
| 24 - 37 | 1687.88 | 1686.87 | 1686.87 | 0.00 | 0 | K.HYKEALEAKERGEK.V (Ions score 89) |
| 24 - 37 | 1687.89 | 1686.88 | 1686.87 | 0.01 | 0 | K.HYKEALEAKERGEK.V (Ions score 112) |
| 24 - 40 | 2030.04 | 2029.04 | 2029.04 | -0.00 | 0 | K.HYKEALEAKERGEKVGW.C (Ions score 96) |
| 24 - 56 | 3794.98 | 3793.97 | 3793.92 | 0.05 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 46) |
| 24 - 56 | 3795.02 | 3794.01 | 3793.92 | 0.09 | 0 | K.HYKEALEAKERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 36) |
| 25 - 34 | 1236.65 | 1235.65 | 1235.65 | -0.00 | 0 | H.YKEALEAKER.G (Ions score 44) |
| 26 - 34 | 1073.58 | 1072.57 | 1072.59 | -0.02 | 0 | Y.KEALEAKER.G (Ions score 18) |
| 26 - 37 | 1387.74 | 1386.73 | 1386.75 | -0.02 | 0 | Y.KEALEAKERGEK.V (Ions score 40) |
| 27 - 34 | 945.49 | 944.49 | 944.49 | -0.01 | 0 | K.EALEAKER.G (Ions score 44) |
| 27 - 34 | 945.50 | 944.50 | 944.49 | 0.00 | 0 | K.EALEAKER.G (Ions score 43) |
| 33 - 56 | 2725.42 | 2724.41 | 2724.37 | 0.04 | 0 | K.ERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 48) |
| 33 - 56 | 2725.45 | 2724.44 | 2724.37 | 0.07 | 0 | K.ERGEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 46) |
| 35 - 56 | 2440.26 | 2439.25 | 2439.23 | 0.02 | 0 | R.GEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 164) |
| 35 - 56 | 2440.30 | 2439.29 | 2439.23 | 0.07 | 0 | R.GEKVGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 157) |
| 38 - 56 | 2021.07 | 2020.06 | 2020.01 | 0.05 | 0 | K.VGWCASNFPQEIATTLGVK.V (Ions score 143) |
| 38 - 56 | 2126.10 | 2125.09 | 2125.07 | 0.02 | 0 | K.VGWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 149) |
| 38 - 70 | 3574.84 | 3573.83 | 3573.82 | 0.01 | 0 | K.VGWCASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 185) |
| 39 - 56 | 2027.00 | 2026.00 | 2026.00 | -0.00 | 0 | V.GWCASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 110) |
| 41 - 53 | 1499.70 | 1498.70 | 1498.71 | -0.02 | 0 | W.CASNFPQEIATTL.G S-pyridylethyl (C) (Ions score 83) |
| 41 - 56 | 1783.90 | 1782.89 | 1782.90 | -0.00 | 0 | W.CASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 101) |
| 41 - 56 | 1783.96 | 1782.95 | 1782.90 | 0.05 | 0 | W.CASNFPQEIATTLGVK.V S-pyridylethyl (C) (Ions score 67) |
| 41 - 70 | 3232.64 | 3231.63 | 3231.65 | -0.02 | 0 | W.CASNFPQEIATTLGVKVVYPENHAAAVAAR.G S-pyridylethyl (C) (Ions score 108) |
| 43 - 56 | 1504.80 | 1503.79 | 1503.79 | -0.00 | 0 | A.SNFPQEIATTLGVK.V (Ions score 69) |
| 43 - 56 | 1504.81 | 1503.80 | 1503.79 | 0.01 | 0 | A.SNFPQEIATTLGVK.V (Ions score 80) |
| 44 - 56 | 1417.77 | 1416.76 | 1416.76 | 0.00 | 0 | S.NFPQEIATTLGVK.V (Ions score 54) |

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|---|---|---|---|---|---|---|---|
| 46 - 53 | 872.47 | 871.46 | 871.47 | -0.00 | 0 | F.PQEIATTL.G | (Ions score 69) |
| 52 - 70 | 1967.05 | 1966.04 | 1966.06 | -0.02 | 0 | T.TLGVKVVYPENHAAAVAAR.G | Deamidation (NQ) (Ions score 27) |
| 54 - 70 | 1751.95 | 1750.94 | 1750.95 | -0.01 | 0 | L.GVKVVYPENHAAAVAAR.G | (Ions score 134) |
| 54 - 70 | 1751.95 | 1750.95 | 1750.95 | -0.00 | 0 | L.GVKVVYPENHAAAVAAR.G | (Ions score 133) |
| 54 - 70 | 1751.96 | 1750.96 | 1750.95 | 0.01 | 0 | L.GVKVVYPENHAAAVAAR.G | (Ions score 139) |
| 54 - 70 | 1752.91 | 1751.91 | 1751.93 | -0.02 | 0 | L.GVKVVYPENHAAAVAAR.G | Deamidation (NQ) (Ions score 16) |
| 55 - 70 | 1694.92 | 1693.91 | 1693.93 | -0.02 | 0 | G.VKVVYPENHAAAVAAR.G | (Ions score 115) |
| 55 - 70 | 1694.92 | 1693.92 | 1693.93 | -0.01 | 0 | G.VKVVYPENHAAAVAAR.G | (Ions score 104) |
| 55 - 70 | 1694.94 | 1693.93 | 1693.93 | 0.00 | 0 | G.VKVVYPENHAAAVAAR.G | (Ions score 126) |
| 56 - 70 | 1595.84 | 1594.84 | 1594.86 | -0.02 | 0 | V.KVVYPENHAAAVAAR.G | (Ions score 48) |
| 57 - 70 | 1467.76 | 1466.75 | 1466.76 | -0.02 | 0 | K.VVYPENHAAAVAAR.G | (Ions score 83) |
| 57 - 70 | 1467.76 | 1466.76 | 1466.76 | -0.01 | 0 | K.VVYPENHAAAVAAR.G | (Ions score 116) |
| 57 - 70 | 1467.81 | 1466.81 | 1466.76 | 0.04 | 0 | K.VVYPENHAAAVAAR.G | (Ions score 121) |
| 57 - 70 | 1467.82 | 1466.81 | 1466.76 | 0.05 | 0 | K.VVYPENHAAAVAAR.G | (Ions score 123) |
| 57 - 70 | 1467.83 | 1466.82 | 1466.76 | 0.06 | 0 | K.VVYPENHAAAVAAR.G | (Ions score 121) |
| 58 - 70 | 1368.69 | 1367.69 | 1367.69 | -0.01 | 0 | V.VYPENHAAAVAAR.G | (Ions score 90) |
| 58 - 70 | 1368.69 | 1367.69 | 1367.69 | -0.01 | 0 | V.VYPENHAAAVAAR.G | (Ions score 66) |
| 58 - 70 | 1368.70 | 1367.69 | 1367.69 | -0.00 | 0 | V.VYPENHAAAVAAR.G | (Ions score 99) |
| 59 - 70 | 1269.62 | 1268.61 | 1268.63 | -0.02 | 0 | V.YPENHAAAVAAR.G | (Ions score 110) |
| 59 - 70 | 1269.62 | 1268.61 | 1268.63 | -0.01 | 0 | V.YPENHAAAVAAR.G | (Ions score 96) |
| 59 - 70 | 1269.63 | 1268.62 | 1268.63 | -0.00 | 0 | V.YPENHAAAVAAR.G | (Ions score 98) |
| 62 - 70 | 880.47 | 879.46 | 879.47 | -0.01 | 0 | E.NHAAAVAAR.G | (Ions score 21) |
| 71 - 81 | 1294.47 | 1293.47 | 1293.49 | -0.02 | 0 | R.GNGQNMCEHAE.A | S-pyridylethyl (C) (Ions score 31) |
| 71 - 85 | 1700.64 | 1699.63 | 1699.65 | -0.02 | 0 | R.GNGQNMCEHAEAMGF.S | S-pyridylethyl (C) (Ions score 72) |
| 71 - 85 | 1701.70 | 1700.69 | 1700.64 | 0.05 | 0 | R.GNGQNMCEHAEAMGF.S | Deamidation (NQ); S-pyridylethyl (C) (Ions score 48) |
| 71 - 92 | 2544.03 | 2543.03 | 2542.98 | 0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGY.A | 2 S-pyridylethyl (C) (Ions score 66) |
| 71 - 94 | 2562.04 | 2561.03 | 2560.98 | 0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ) (Ions score 12) |
| 71 - 94 | 2666.08 | 2665.07 | 2665.06 | 0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 121) |
| 71 - 94 | 2666.09 | 2665.08 | 2665.06 | 0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 143) |
| 71 - 94 | 2666.98 | 2665.98 | 2666.04 | -0.06 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); S-pyridylethyl (C) (Ions score 46) |
| 71 - 94 | 2666.99 | 2665.98 | 2666.04 | -0.06 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); S-pyridylethyl (C) (Ions score 29) |
| 71 - 94 | 2667.08 | 2666.07 | 2666.04 | 0.03 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); S-pyridylethyl (C) (Ions score 110) |
| 71 - 94 | 2771.12 | 2770.11 | 2770.11 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 55) |
| 71 - 94 | 2771.14 | 2770.13 | 2770.11 | 0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 57) |
| 71 - 94 | 2771.16 | 2770.15 | 2770.11 | 0.03 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 153) |
| 71 - 94 | 2771.17 | 2770.17 | 2770.11 | 0.05 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 S-pyridylethyl (C) (Ions score 153) |
| 71 - 94 | 2772.12 | 2771.11 | 2771.10 | 0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); 2 S-pyridylethyl (C) (Ions score 136) |
| 71 - 94 | 2787.11 | 2786.10 | 2786.11 | -0.01 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 42) |
| 71 - 94 | 2787.14 | 2786.13 | 2786.11 | 0.02 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 39) |
| 71 - 94 | 2787.15 | 2786.14 | 2786.11 | 0.03 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | Oxidation (M); 2 S-pyridylethyl (C) (Ions score 65) |
| 71 - 94 | 2803.11 | 2802.11 | 2802.10 | 0.00 | 0 | R.GNGQNMCEHAEAMGFSNDVCGYAR.V | 2 Oxidation (M); 2 S-pyridylethyl (C) (Ions score 25) |
| 74 - 94 | 2559.04 | 2558.04 | 2557.98 | 0.06 | 0 | G.QNMCEHAEAMGFSNDVCGYAR.V | Deamidation (NQ); 2 Oxidation (M); Pyro-glu (N-term Q); 2 S-pyridylethyl (C) (Ions score 9) |
| 84 - 92 | 1066.41 | 1065.40 | 1065.42 | -0.02 | 0 | M.GFSNDVCGY.A | S-pyridylethyl (C) (Ions score 36) |
| 84 - 94 | 1293.55 | 1292.55 | 1292.56 | -0.01 | 0 | M.GFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 55) |
| 84 - 94 | 1293.56 | 1292.55 | 1292.56 | -0.01 | 0 | M.GFSNDVCGYAR.V | S-pyridylethyl (C) (Ions score 63) |
| 86 - 94 | 1089.48 | 1088.48 | 1088.47 | 0.00 | 0 | F.SNDVCGYAR.V | S-pyridylethyl (C) (Ions score 39) |
| 86 - 94 | 1089.50 | 1088.49 | 1088.47 | 0.02 | 0 | F.SNDVCGYAR.V | S-pyridylethyl (C) (Ions score 38) |
| 136 - 158 | 2661.37 | 2660.36 | 2660.34 | 0.02 | 0 | K.TLDIPMILIDIPYNTENTVSQDR.I | (Ions score 182) |
| 136 - 158 | 2661.42 | 2660.42 | 2660.34 | 0.08 | 0 | K.TLDIPMILIDIPYNTENTVSQDR.I | (Ions score 161) |
| 136 - 160 | 2902.51 | 2901.50 | 2901.52 | -0.01 | 0 | K.TLDIPMILIDIPYNTENTVSQDRIK.Y | (Ions score 89) |
| 164 - 171 | 907.44 | 906.44 | 906.44 | -0.01 | 0 | R.AQFDDAIK.Q | (Ions score 55) |
| 164 - 171 | 907.45 | 906.44 | 906.44 | -0.01 | 0 | R.AQFDDAIK.Q | (Ions score 62) |
| 164 - 179 | 1805.93 | 1804.92 | 1804.92 | 0.00 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 113) |
| 164 - 179 | 1805.94 | 1804.94 | 1804.92 | 0.02 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 103) |
| 164 - 179 | 1805.95 | 1804.94 | 1804.92 | 0.02 | 0 | R.AQFDDAIKQLEEITGK.K | (Ions score 104) |
| 164 - 180 | 1934.07 | 1933.06 | 1933.02 | 0.05 | 0 | R.AQFDDAIKQLEEITGKK.W | (Ions score 105) |
| 164 - 180 | 1934.07 | 1933.07 | 1933.02 | 0.05 | 0 | R.AQFDDAIKQLEEITGKK.W | (Ions score 107) |
| 164 - 181 | 2120.09 | 2119.09 | 2119.09 | -0.01 | 0 | R.AQFDDAIKQLEEITGKKW.D | (Ions score 55) |
| 164 - 191 | 3369.71 | 3368.70 | 3368.66 | 0.04 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 151) |
| 164 - 191 | 3369.71 | 3368.70 | 3368.66 | 0.05 | 0 | R.AQFDDAIKQLEEITGKKWDENKFEEVMK.I | (Ions score 83) |
| 171 - 179 | 1045.59 | 1044.58 | 1044.58 | 0.00 | 0 | I.KQLEEITGK.K | (Ions score 33) |
| 172 - 191 | 2481.27 | 2480.26 | 2480.23 | 0.04 | 0 | K.QLEEITGKKWDENKFEEVMK.I | (Ions score 90) |
| 180 - 191 | 1582.75 | 1581.74 | 1581.75 | -0.01 | 0 | K.KWDENKFEEVMK.I | (Ions score 71) |
| 180 - 191 | 1582.75 | 1581.74 | 1581.75 | -0.01 | 0 | K.KWDENKFEEVMK.I | (Ions score 73) |
| 180 - 191 | 1582.76 | 1581.75 | 1581.75 | 0.00 | 0 | K.KWDENKFEEVMK.I | (Ions score 93) |
| 180 - 198 | 2326.21 | 2325.20 | 2325.13 | 0.07 | 0 | K.KWDENKFEEVMKISQESAK.Q | (Ions score 89) |
| 180 - 202 | 2909.46 | 2908.45 | 2908.45 | -0.00 | 0 | K.KWDENKFEEVMKISQESAKQWLR.A | (Ions score 199) |
| 180 - 202 | 2909.54 | 2908.53 | 2908.45 | 0.08 | 0 | K.KWDENKFEEVMKISQESAKQWLR.A | (Ions score 207) |
| 181 - 191 | 1454.64 | 1453.63 | 1453.65 | -0.02 | 0 | K.WDENKFEEVMK.I | (Ions score 69) |
| 181 - 191 | 1454.65 | 1453.64 | 1453.65 | -0.01 | 0 | K.WDENKFEEVMK.I | (Ions score 74) |
| 181 - 191 | 1454.66 | 1453.66 | 1453.65 | 0.00 | 0 | K.WDENKFEEVMK.I | (Ions score 43) |
| 192 - 200 | 1076.52 | 1075.51 | 1075.53 | -0.02 | 0 | K.ISQESAKQW.L | (Ions score 48) |

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|---|---|---|---|---|---|---|
| 192 - 202 | 1345.77 | 1344.76 | 1344.71 | 0.05 | 0 | K.ISQESAKQWLR.A (Ions score 97) |
| 209 - 230 | 2457.18 | 2456.17 | 2456.18 | -0.00 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G (Ions score 101) |
| 209 - 230 | 2457.25 | 2456.24 | 2456.18 | 0.07 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G (Ions score 116) |
| 209 - 230 | 2562.25 | 2561.24 | 2561.23 | 0.01 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G S-pyridylethyl (C) (Ions score 157) |
| 209 - 230 | 2562.32 | 2561.31 | 2561.23 | 0.07 | 0 | K.YKPSPFSGFDLFNHMAVAVCAR.G S-pyridylethyl (C) (Ions score 136) |
| 215 - 230 | 1842.86 | 1841.86 | 1841.87 | -0.01 | 0 | F.SGFDLFNHMAVAVCAR.G S-pyridylethyl (C) (Ions score 103) |
| 218 - 230 | 1551.76 | 1550.75 | 1550.75 | -0.00 | 0 | F.DLFNHMAVAVCAR.G S-pyridylethyl (C) (Ions score 92) |
| 221 - 230 | 1176.57 | 1175.56 | 1175.57 | -0.01 | 0 | F.NHMAVAVCAR.G S-pyridylethyl (C) (Ions score 66) |
| 231 - 240 | 1037.48 | 1036.47 | 1036.48 | -0.01 | 0 | R.GTQEAADAFK.M (Ions score 71) |
| 231 - 240 | 1037.49 | 1036.49 | 1036.48 | 0.00 | 0 | R.GTQEAADAFK.M (Ions score 71) |
| 231 - 254 | 2645.29 | 2644.28 | 2644.23 | 0.05 | 0 | R.GTQEAADAFKMLADEYEENVKTGK.S (Ions score 173) |
| 231 - 254 | 2645.34 | 2644.33 | 2644.23 | 0.10 | 0 | R.GTQEAADAFKMLADEYEENVKTGK.S (Ions score 176) |
| 241 - 251 | 1340.59 | 1339.58 | 1339.60 | -0.02 | 0 | K.MLADEYEENVK.T (Ions score 72) |
| 241 - 251 | 1340.59 | 1339.58 | 1339.60 | -0.01 | 0 | K.MLADEYEENVK.T (Ions score 60) |
| 241 - 251 | 1340.59 | 1339.59 | 1339.60 | -0.01 | 0 | K.MLADEYEENVK.T (Ions score 72) |
| 241 - 254 | 1626.76 | 1625.75 | 1625.76 | -0.01 | 0 | K.MLADEYEENVKTGK.S (Ions score 102) |
| 241 - 254 | 1626.76 | 1625.75 | 1625.76 | -0.01 | 0 | K.MLADEYEENVKTGK.S (Ions score 100) |
| 241 - 254 | 1626.79 | 1625.78 | 1625.76 | 0.02 | 0 | K.MLADEYEENVKTGK.S (Ions score 103) |
| 252 - 262 | 1255.62 | 1254.61 | 1254.62 | -0.01 | 0 | K.TGKSTYRGEEK.Q (Ions score 14) |
| 252 - 262 | 1255.62 | 1254.61 | 1254.62 | -0.01 | 0 | K.TGKSTYRGEEK.Q (Ions score 22) |
| 252 - 264 | 1539.76 | 1538.75 | 1538.78 | -0.03 | 0 | K.TGKSTYRGEEKQR.I (Ions score 67) |
| 252 - 264 | 1539.78 | 1538.77 | 1538.78 | -0.01 | 0 | K.TGKSTYRGEEKQR.I (Ions score 67) |
| 254 - 264 | 1381.70 | 1380.70 | 1380.71 | -0.01 | 0 | G.KSTYRGEEKQR.I (Ions score 32) |
| 254 - 264 | 1381.71 | 1380.70 | 1380.71 | -0.01 | 0 | G.KSTYRGEEKQR.I (Ions score 33) |
| 255 - 262 | 969.46 | 968.45 | 968.46 | -0.01 | 0 | K.STYRGEEK.Q (Ions score 38) |
| 255 - 263 | 1097.56 | 1096.56 | 1096.51 | 0.04 | 0 | K.STYRGEEKQ.R (Ions score 23) |
| 255 - 264 | 1253.62 | 1252.61 | 1252.62 | -0.01 | 0 | K.STYRGEEKQR.I (Ions score 57) |
| 255 - 264 | 1253.63 | 1252.62 | 1252.62 | 0.01 | 0 | K.STYRGEEKQR.I (Ions score 58) |
| 255 - 264 | 1253.64 | 1252.63 | 1252.62 | 0.02 | 0 | K.STYRGEEKQR.I (Ions score 61) |
| 256 - 264 | 1166.58 | 1165.57 | 1165.58 | -0.01 | 0 | S.TYRGEEKQR.I (Ions score 15) |
| 265 - 277 | 1580.82 | 1579.81 | 1579.82 | -0.01 | 0 | R.ILFEGIACWPYLR.H (Ions score 93) |
| 265 - 277 | 1580.85 | 1579.84 | 1579.82 | 0.02 | 0 | R.ILFEGIACWPYLR.H (Ions score 78) |
| 265 - 277 | 1685.87 | 1684.86 | 1684.88 | -0.02 | 0 | R.ILFEGIACWPYLR.H S-pyridylethyl (C) (Ions score 75) |
| 265 - 277 | 1685.87 | 1684.87 | 1684.88 | -0.01 | 0 | R.ILFEGIACWPYLR.H S-pyridylethyl (C) (Ions score 30) |
| 265 - 277 | 1685.91 | 1684.91 | 1684.88 | 0.03 | 0 | R.ILFEGIACWPYLR.H S-pyridylethyl (C) (Ions score 78) |
| 266 - 277 | 1572.80 | 1571.80 | 1571.80 | 0.00 | 0 | I.LFEGIACWPYLR.H S-pyridylethyl (C) (Ions score 35) |
| 266 - 277 | 1572.82 | 1571.81 | 1571.80 | 0.02 | 0 | I.LFEGIACWPYLR.H S-pyridylethyl (C) (Ions score 36) |
| 267 - 277 | 1459.70 | 1458.69 | 1458.71 | -0.02 | 0 | L.FEGIACWPYLR.H S-pyridylethyl (C) (Ions score 41) |
| 267 - 277 | 1459.70 | 1458.70 | 1458.71 | -0.02 | 0 | L.FEGIACWPYLR.H S-pyridylethyl (C) (Ions score 43) |
| 316 - 327 | 1318.69 | 1317.69 | 1317.69 | -0.01 | 0 | K.VPNSISFENALK.M (Ions score 90) |
| 316 - 327 | 1318.70 | 1317.69 | 1317.69 | -0.00 | 0 | K.VPNSISFENALK.M (Ions score 100) |
| 316 - 329 | 1605.84 | 1604.83 | 1604.83 | -0.00 | 0 | K.VPNSISFENALKMR.L (Ions score 33) |
| 328 - 347 | 2197.15 | 2196.14 | 2196.14 | -0.00 | 0 | K.MRLNAVTSTNTEGAVIHINR.S (Ions score 35) |
| 328 - 347 | 2197.15 | 2196.14 | 2196.14 | -0.00 | 0 | K.MRLNAVTSTNTEGAVIHINR.S (Ions score 83) |
| 330 - 347 | 1910.00 | 1908.99 | 1909.00 | -0.01 | 0 | R.LNAVTSTNTEGAVIHINR.S (Ions score 65) |
| 330 - 347 | 1910.00 | 1908.99 | 1909.00 | -0.01 | 0 | R.LNAVTSTNTEGAVIHINR.S (Ions score 65) |
| 330 - 347 | 1910.01 | 1909.00 | 1909.00 | 0.00 | 0 | R.LNAVTSTNTEGAVIHINR.S (Ions score 134) |
| 330 - 347 | 1910.02 | 1909.02 | 1909.00 | 0.01 | 0 | R.LNAVTSTNTEGAVIHINR.S (Ions score 145) |
| 330 - 347 | 1910.03 | 1909.02 | 1909.00 | 0.02 | 0 | R.LNAVTSTNTEGAVIHINR.S (Ions score 166) |
| 351 - 361 | 1354.71 | 1353.70 | 1353.71 | -0.01 | 0 | K.LWSGFLYELAR.R (Ions score 75) |
| 351 - 361 | 1354.71 | 1353.70 | 1353.71 | -0.01 | 0 | K.LWSGFLYELAR.R (Ions score 99) |
| 351 - 361 | 1354.75 | 1353.74 | 1353.71 | 0.03 | 0 | K.LWSGFLYELAR.R (Ions score 98) |
| 352 - 361 | 1241.62 | 1240.61 | 1240.62 | -0.01 | 0 | L.WSGFLYELAR.R (Ions score 84) |
| 352 - 361 | 1241.64 | 1240.64 | 1240.62 | 0.01 | 0 | L.WSGFLYELAR.R (Ions score 84) |
| 362 - 380 | 2076.03 | 2075.02 | 2075.02 | 0.01 | 0 | R.RLEKETGIPVVSFDGDQAD.P (Ions score 118) |
| 362 - 382 | 2329.20 | 2328.19 | 2328.17 | 0.02 | 0 | R.RLEKETGIPVVSFDGDQADPR.N (Ions score 72) |
| 362 - 382 | 2329.22 | 2328.22 | 2328.17 | 0.05 | 0 | R.RLEKETGIPVVSFDGDQADPR.N (Ions score 82) |
| 362 - 382 | 2329.23 | 2328.22 | 2328.17 | 0.05 | 0 | R.RLEKETGIPVVSFDGDQADPR.N (Ions score 62) |
| 363 - 382 | 2173.09 | 2172.08 | 2172.07 | 0.01 | 0 | R.LEKETGIPVVSFDGDQADPR.N (Ions score 129) |
| 363 - 382 | 2173.09 | 2172.08 | 2172.07 | 0.01 | 0 | R.LEKETGIPVVSFDGDQADPR.N (Ions score 157) |
| 363 - 382 | 2173.10 | 2172.09 | 2172.07 | 0.02 | 0 | R.LEKETGIPVVSFDGDQADPR.N (Ions score 154) |
| 366 - 382 | 1802.87 | 1801.86 | 1801.85 | 0.01 | 0 | K.ETGIPVVSFDGDQADPR.N (Ions score 139) |
| 366 - 382 | 1802.87 | 1801.87 | 1801.85 | 0.02 | 0 | K.ETGIPVVSFDGDQADPR.N (Ions score 136) |
| 366 - 382 | 1802.88 | 1801.87 | 1801.85 | 0.02 | 0 | K.ETGIPVVSFDGDQADPR.N (Ions score 141) |
| 366 - 384 | 2063.98 | 2062.97 | 2062.96 | 0.01 | 0 | K.ETGIPVVSFDGDQADPRNF.S (Ions score 58) |
| 367 - 382 | 1673.82 | 1672.81 | 1672.81 | 0.00 | 0 | E.TGIPVVSFDGDQADPR.N (Ions score 20) |
| 367 - 382 | 1673.82 | 1672.81 | 1672.81 | 0.01 | 0 | E.TGIPVVSFDGDQADPR.N (Ions score 30) |
| 373 - 382 | 1107.45 | 1106.45 | 1106.46 | -0.02 | 0 | V.SFDGDQADPR.N (Ions score 31) |
| 373 - 382 | 1107.46 | 1106.45 | 1106.46 | -0.01 | 0 | V.SFDGDQADPR.N (Ions score 50) |
| 374 - 382 | 1020.43 | 1019.42 | 1019.43 | -0.01 | 0 | S.FDGDQADPR.N (Ions score 41) |
| 374 - 382 | 1020.43 | 1019.43 | 1019.43 | -0.00 | 0 | S.FDGDQADPR.N (Ions score 30) |
| 383 - 392 | 1230.53 | 1229.52 | 1229.53 | -0.01 | 0 | R.NFSEAQYDTR.I (Ions score 62) |
| 383 - 392 | 1230.53 | 1229.52 | 1229.53 | -0.01 | 0 | R.NFSEAQYDTR.I (Ions score 79) |
| 383 - 392 | 1230.55 | 1229.54 | 1229.53 | 0.01 | 0 | R.NFSEAQYDTR.I (Ions score 87) |
| 383 - 392 | 1231.56 | 1230.55 | 1230.52 | 0.04 | 0 | R.NFSEAQYDTR.I Deamidation (NQ) (Ions score 37) |
| 383 - 404 | 2541.34 | 2540.33 | 2540.27 | 0.06 | 0 | R.NFSEAQYDTRIQGLNEVMVAKK.E (Ions score 34) |
| 383 - 407 | 2870.44 | 2869.43 | 2869.39 | 0.04 | 0 | R.NFSEAQYDTRIQGLNEVMVAKKEAE.- (Ions score 64) |
| 384 - 392 | 1116.48 | 1115.47 | 1115.49 | -0.02 | 0 | N.FSEAQYDTR.I (Ions score 64) |
| 384 - 392 | 1116.49 | 1115.48 | 1115.49 | -0.00 | 0 | N.FSEAQYDTR.I (Ions score 35) |
| 384 - 392 | 1116.51 | 1115.50 | 1115.49 | 0.02 | 0 | N.FSEAQYDTR.I (Ions score 66) |
| 384 - 392 | 1116.51 | 1115.51 | 1115.49 | 0.02 | 0 | N.FSEAQYDTR.I (Ions score 68) |
| 385 - 392 | 969.42 | 968.41 | 968.42 | -0.01 | 0 | F.SEAQYDTR.I (Ions score 46) |
| 385 - 392 | 969.42 | 968.42 | 968.42 | -0.00 | 0 | F.SEAQYDTR.I (Ions score 44) |
| 386 - 392 | 882.38 | 881.38 | 881.39 | -0.01 | 0 | S.EAQYDTR.I (Ions score 23) |
| 393 - 403 | 1201.65 | 1200.64 | 1200.65 | -0.02 | 0 | R.IQGLNEVMVAK.K (Ions score 30) |
| 393 - 403 | 1201.65 | 1200.64 | 1200.65 | -0.01 | 0 | R.IQGLNEVMVAK.K (Ions score 69) |
| 393 - 404 | 1329.74 | 1328.73 | 1328.75 | -0.02 | 0 | R.IQGLNEVMVAKK.E (Ions score 53) |
| 393 - 404 | 1329.74 | 1328.73 | 1328.75 | -0.01 | 0 | R.IQGLNEVMVAKK.E (Ions score 32) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 393 - 404 | 1329.74 | 1328.74 | 1328.75 | -0.01 | 0 | R.IQGLNEVMVAKK.E | (Ions score 32) |
| 393 - 404 | 1329.75 | 1328.74 | 1328.75 | -0.01 | 0 | R.IQGLNEVMVAKK.E | (Ions score 87) |
| 393 - 404 | 1329.76 | 1328.75 | 1328.75 | 0.00 | 0 | R.IQGLNEVMVAKK.E | (Ions score 60) |
| 393 - 407 | 1658.86 | 1657.85 | 1657.87 | -0.02 | 0 | R.IQGLNEVMVAKKEAE.- | (Ions score 78) |

**Protein View of the small subunit FldC of the recombinant dehydratase**

Match to: **R-phenyllactate-small-su** Score: **11228**
**dehydratase small subunit [Clostridium sporogenes]**

Nominal mass ($M_r$): **43120**; Calculated pI value: **5.17**
NCBI BLAST search of R-phenyllactate-small-su against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Deamidation (NQ),Oxidation (M),Pyro-glu (N-term E),Pyro-glu (N-term Q),S-pyridylethyl (C)
No enzyme cleavage specificity
Sequence Coverage: **88%**

Matched peptides shown in **Bold Red**

```
   1 MSNSDKFFND FKDIVENPKK YIMKHMEQTG QKAIGCMPLY TPEELVLAAG
  51 MFPVGVWGSN TELSKAKTYF PAFICSILQT TLENALNGEY DMLSGMMITN
 101 YCDSLKCMGQ NFKLTVENIE FIPVTVPQNR KMEAGKEFLK SQYKMNIEQL
 151 EKISGNKITD ESLEKAIEIY DEHRKVMNDF SMLASKYPGI ITPTKRNYVM
 201 KSAYYMDKKE HTEKVRQLMD EIKAIEPKPF EGKRVITTGI IADSEDLLKI
 251 LEENNIAIVG DDIAHESRQY RTLTPEANTP MDRLAEQFAN RECSTLYDPE
 301 KKRGQYIVEM AKERKADGII FFMTKFCDPE EYDYPQMKKD FEEAGIPHVL
 351 IETDMQMKNY EQARTAIQAF SETL
```

# *ERKLÄRUNG*

Hiermit versichere ich, dass ich mine Dissertation mit dem Titel:

(*R*)-Indolelactyl-CoA dehydratase, the key enzyme of tryptophan reduction to indolepropionate in *Clostridium sporogenes*

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die vorliegende Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen deutschen Hochschule eingereicht und hat noch keinen sonstigen Prüfung Zwecken gedient.

(Ort, Datum) Unterschrift